



(11) **EP 3 102 684 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
06.05.2020 Bulletin 2020/19

(21) Application number: **15708059.9**

(22) Date of filing: **06.02.2015**

(51) Int Cl.:
C12N 15/82^(2006.01) C07K 14/415^(2006.01)

(86) International application number:
PCT/US2015/014816

(87) International publication number:
WO 2015/120270 (13.08.2015 Gazette 2015/32)

(54) **INSECTICIDAL PROTEINS AND METHODS FOR THEIR USE**

INSEKTIZIDPROTEINE UND VERFAHREN ZU DEREN VERWENDUNG

PROTÉINES INSECTICIDES ET LEURS PROCÉDÉS D'UTILISATION

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **07.02.2014 US 201461937288 P**

(43) Date of publication of application:
14.12.2016 Bulletin 2016/50

(73) Proprietors:
• **Pioneer Hi-Bred International, Inc.**
Johnston, Iowa 50131-1014 (US)
• **E. I. du Pont de Nemours and Company**
Wilmington, DE 19805 (US)

(72) Inventors:
• **BARRY, Jennifer**
Ames, Iowa 50014 (US)
• **HAYES, Kevin**
Urbandale, Iowa 50323 (US)
• **LIU, Lu**
Palo Alto, California 94301 (US)
• **SCHEPERS, Eric**
Port Deposit, Maryland 21904 (US)
• **YALPANI, Nasser**
Johnston, Iowa 50131 (US)

(74) Representative: **J A Kemp LLP**
14 South Square
Gray's Inn
London WC1R 5JJ (GB)

(56) References cited:
WO-A2-2013/098858

- **H. K. ABICHT ET AL: "Genome Sequence of Desulfosporosinus sp. OT, an Acidophilic Sulfate-Reducing Bacterium from Copper Mining Waste in Norilsk, Northern Siberia", JOURNAL OF BACTERIOLOGY, vol. 193, no. 21, 1 November 2011 (2011-11-01), pages 6104-6105, XP055196331, ISSN: 0021-9193, DOI: 10.1128/JB.06018-11 -& DATABASE UniProt [Online] 16 November 2011 (2011-11-16), "SubName: Full=Uncharacterized protein {ECO:0000313|EMBL:EGW36042.1};", XP002741068, retrieved from EBI accession no. UNIPROT:G2G258 Database accession no. G2G258**
- **TAMURA SAKI ET AL: "Purification, characterization and cDNA cloning of two natterin-like toxins from the skin secretion of oriental catfish *Plotosus lineatus*", TOXICON, vol. 58, no. 5, October 2011 (2011-10), pages 430-438, XP28314820, ISSN: 0041-0101**

EP 3 102 684 B1

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description**FIELD**

5 **[0001]** This disclosure relates to the field of molecular biology. Provided are novel genes that encode pesticidal proteins. These pesticidal proteins and the nucleic acid sequences that encode them are useful in preparing pesticidal formulations and in the production of transgenic pest-resistant plants.

BACKGROUND

10 **[0002]** Biological control of insect pests of agricultural significance using a microbial agent, such as fungi, bacteria or another species of insect affords an environmentally friendly and commercially attractive alternative to synthetic chemical pesticides. Generally speaking, the use of biopesticides presents a lower risk of pollution and environmental hazards and biopesticides provide greater target specificity than is characteristic of traditional broad-spectrum chemical insecticides. In addition, biopesticides often cost less to produce and thus improve economic yield for a wide variety of crops.

15 **[0003]** Certain species of microorganisms of the genus *Bacillus* are known to possess pesticidal activity against a range of insect pests including Lepidoptera, Diptera, Coleoptera, Hemiptera and others. *Bacillus thuringiensis* (*Bt*) and *Bacillus popilliae* are among the most successful biocontrol agents discovered to date. Insect pathogenicity has also been attributed to strains of *B. larvae*, *B. lentimorbus*, *B. sphaericus* and *B. cereus*. Microbial insecticides, particularly those obtained from *Bacillus* strains, have played an important role in agriculture as alternatives to chemical pest control.

20 **[0004]** Crop plants have been developed with enhanced insect resistance by genetically engineering crop plants to produce pesticidal proteins from *Bacillus*. For example, corn and cotton plants have been genetically engineered to produce pesticidal proteins isolated from strains of *Bt*. These genetically engineered crops are now widely used in agriculture and have provided the farmer with an environmentally friendly alternative to traditional insect-control methods. While they have proven to be very successful commercially, these genetically engineered, insect-resistant crop plants provide resistance to only a narrow range of the economically important insect pests. In some cases, insects can develop resistance to different insecticidal compounds, which raises the need to identify alternative biological control agents for pest control.

25 **[0005]** Accordingly, there remains a need for new pesticidal proteins with different ranges of insecticidal activity against insect pests, e.g., insecticidal proteins which are active against a variety of insects in the order Lepidoptera and the order Coleoptera including but not limited to insect pests that have developed resistance to existing insecticides.

30 **[0006]** WO2013/098858 A2 discloses a novel insecticidal chitinase protein, its encoding nucleotide and application thereof.

SUMMARY

35 **[0007]** Compositions and methods for conferring pesticidal activity to bacteria, plants, plant cells, tissues and seeds are provided. Compositions include nucleic acid molecules encoding sequences for pesticidal and insecticidal polypeptides, vectors comprising those nucleic acid molecules, and host cells comprising the vectors. Compositions also include the pesticidal polypeptide sequences and antibodies to those polypeptides. The nucleic acid sequences can be used in DNA constructs or expression cassettes for transformation and expression in organisms, including microorganisms and plants. The nucleotide or amino acid sequences may be synthetic sequences that have been designed for expression in an organism including, but not limited to, a microorganism or a plant. Compositions also comprise transformed bacteria, plants, plant cells, tissues and seeds.

40 **[0008]** The invention provides a recombinant PtIP-50 polypeptide, wherein the amino acid sequence of the PtIP-50 polypeptide has:

- a) at least 80% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Aa as shown in Figures 10a-10o;
- 50 b) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, or PtIP-50Bd as shown in Figures 10a-10o;
- c) at least 70% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Fb, PtIP-50Fs, PtIP-50Fo, PtIP-50Fk, PtIP-50Fe, PtIP-50FI, PtIP-50Fn, or PtIP-50Ft as shown in Figures 10a-10o;
- 55 d) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Fd, PtIP-50Fm, PtIP-50Fr, PtIP-50Fg, or PtIP-50Fh as shown in Figures 10a-10o;
- e) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, or PtIP-50Gd as shown in Figures 10a-10o; or

f) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Fa, PtIP-50Ff, PtIP-50Fq, PtIP-50Fi, or PtIP-50Fj as shown in Figures 10a-10o;

wherein the PtIP-50 polypeptide has insecticidal activity in combination with a PtIP-65 polypeptide as defined below.

[0009] The invention further provides a recombinant PtIP-65 polypeptide, wherein the amino acid sequence of the PtIP-65 polypeptide has:

a) at least 80% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Aa as shown in Figures 17a-17k;

b) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, or PtIP-65Ca as shown in Figures 17a-17k;

c) at least 70% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Fa or PtIP-65Fb as shown in Figures 17a-17k;

d) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Ha, PtIP-65Gd, or PtIP-65Ge as shown in Figures 17a-17k;

e) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Hh or PtIP-65Hg as shown in Figures 17a-17k;

f) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, or PtIP-65Hf as shown in Figures 17a-17k; or

g) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Hj or PtIP-65Hk as shown in Figures 17a-17k;

wherein the PtIP-65 polypeptide has insecticidal activity in combination with a PtIP-50 polypeptide as defined above.

[0010] Yet further provided by the invention is a polynucleotide encoding the PtIP-50 polypeptide or the PtIP-65 polypeptide as defined above.

[0011] The invention further provides a composition comprising the PtIP-50 polypeptide as defined above and the PtIP-65 polypeptide as defined above.

[0012] Yet further provided by the invention is a fusion protein comprising:

a) the PtIP-50 polypeptide as defined above;

b) the PtIP-65 polypeptide as defined above; or

c) the PtIP-50 polypeptide as defined above and the PtIP-65 polypeptide as defined above.

[0013] The invention further provides a method:

a) for controlling an insect pest population, comprising contacting the insect pest population with an insecticidally-effective amount of the PtIP-50 polypeptide as defined above and the PtIP-65 polypeptide as defined above;

b) of inhibiting growth of or killing an insect pest, comprising contacting the insect pest with a composition comprising an insecticidally-effective amount of the PtIP-50 polypeptide as defined above and the PtIP-65 polypeptide as defined above; or

c) of controlling Lepidoptera and/or Coleoptera insect infestation in a transgenic plant and providing insect resistance management, comprising expressing in the plant an insecticidally-effective amount of the PtIP-50 polypeptide as defined above and the PtIP-65 polypeptide as defined above;

preferably wherein the insect or insect population is resistant to at least one Bt toxin.

[0014] Yet further provided by the invention is the use of the PtIP-50 polypeptide as defined above and the PtIP-65 polypeptide as defined above to inhibit growth or kill an insect or insect population.

[0015] The invention further provides a transgenic plant or plant cell comprising the polynucleotide or polynucleotides as defined above.

[0016] Yet further provided by the invention is a DNA construct comprising the polynucleotide or polynucleotides as defined above;

preferably wherein the DNA construct further comprises at least one heterologous regulatory sequence operably linked to the polynucleotide.

[0017] The invention further provides a transgenic plant or plant cell stably transfected with the DNA construct as defined above.

[0018] Yet further provided by the invention is a method:

a) for controlling an insect pest population, comprising contacting the insect pest population with the transgenic

plant as defined above; or

b) of inhibiting growth of or killing an insect pest population, comprising contacting the insect pest population with the transgenic plant as defined above.

5 **[0019]** Isolated or recombinant nucleic acid molecules are provided encoding *Pteridophyta* Insecticidal Protein-50 (PtIP-50) polypeptides including amino acid substitutions, deletions, insertions, fragments thereof, and combinations thereof and *Pteridophyta* Insecticidal Protein-65 (PtIP-65) polypeptides including amino acid substitutions, deletions, insertions, fragments thereof, and combinations thereof. Additionally, amino acid sequences corresponding to the PtIP-50 polypeptides and PtIP-65 polypeptides are encompassed. Provided are isolated or recombinant nucleic acid molecules capable of encoding PtIP-50 polypeptides as well as amino acid substitutions, deletions, insertions, fragments thereof, and combinations thereof. Nucleic acid sequences that are complementary to a nucleic acid sequence of the embodiments or that hybridize to a sequence of the embodiments are also disclosed. Also provided are isolated or recombinant PtIP-50 polypeptides as well as amino acid substitutions, deletions, insertions, fragments thereof and combinations thereof. Provided are isolated or recombinant nucleic acid molecules capable of encoding PtIP-65 polypeptides as well as amino acid substitutions, deletions, insertions, fragments thereof, and combinations thereof. Nucleic acid sequences that are complementary to a nucleic acid sequence of the embodiments or that hybridize to a sequence of the embodiments are also disclosed. Also provided are isolated or recombinant PtIP-65 polypeptides as well as amino acid substitutions, deletions, insertions, fragments thereof and combinations thereof.

10 **[0020]** Methods are provided for producing the polypeptides and for using those polypeptides for controlling or killing a Lepidopteran, Coleopteran, nematode, fungi, and/or Dipteran pests. The transgenic plants of the embodiments express one or more of the pesticidal sequences disclosed herein. In various embodiments, the transgenic plant further comprises one or more additional genes for insect resistance, for example, one or more additional genes for controlling Coleopteran, Lepidopteran, Hemipteran or nematode pests. It will be understood by one of skill in the art that the transgenic plant may comprise any gene imparting an agronomic trait of interest.

15 **[0021]** Methods for detecting the nucleic acids and polypeptides of the embodiments in a sample are also disclosed. A kit for detecting the presence of a PtIP-50 polypeptide and/or a PtIP-65 polypeptide or detecting the presence of a polynucleotide encoding a PtIP-50 polypeptide and/or a PtIP-65 polypeptide in a sample is disclosed. The kit may be provided along with all reagents and control samples necessary for carrying out a method for detecting the intended agent, as well as instructions for use.

20 **[0022]** The compositions and methods of the embodiments are useful for the production of organisms with enhanced pest resistance or tolerance. These organisms and compositions comprising the organisms are desirable for agricultural purposes. The compositions of the embodiments are also useful for generating altered or improved proteins that have pesticidal activity or for detecting the presence of PtIP-50 polypeptides or nucleic acids and PtIP-65 polypeptides or nucleic acids in products or organisms.

35 BRIEF DESCRIPTION OF THE FIGURES

[0023]

40 Figure 1 the phylogeny of ferns based on the classification for extant ferns by A. R. Smith et al, TAXON, 55:705-731 (2006).

Figure 2 shows the phylogeny tree of the PtIP-65 polypeptides: PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, PtIP-65Ca, PtIP-65Fa, PtIP-65Fb, PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Gd, PtIP-65Ge, PtIP-65Ha, PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, PtIP-65Hf, PtIP-65Hg, PtIP-65Hh, PtIP-65Hj, and PtIP-65Hk.

45 Figures 3a - 3i show an alignment of the amino acid sequences of PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, PtIP-65Ca, PtIP-65Fa, PtIP-65Fb, PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Gd, PtIP-65Ge, PtIP-65Ha, PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, PtIP-65Hf, PtIP-65Hg, PtIP-65Hh, PtIP-65Hj, and PtIP-65Hk. The amino acid diversity is indicated with shading.

50 Figures 4a - 4b show an alignment of the amino acid sequences of PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, and PtIP-65Ca. The amino acid diversity between PtIP-65Aa and PtIP-65Ba, PtIP-65Bb, and PtIP-65Ca is indicated with shading.

Figures 5a - 5c show an alignment of the amino acid sequences of PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Gd, PtIP-65Ge, PtIP-65Ha. The amino acid diversity between PtIP-65Ga and PtIP-65Gb, PtIP-65Gc, PtIP-65Gd, PtIP-65Ge, and PtIP-65Ha is indicated with shading.

55 Figures 6a - 6c show an alignment of the amino acid sequences of PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, and PtIP-65Hf. The amino acid diversity between PtIP-65Hb and PtIP-65Hc, PtIP-65Hd, PtIP-65He, and PtIP-65Hf is indicated with shading.

Figure 7 shows an alignment of the amino acid sequences of PtIP-65Fa and PtIP-65Fb. The amino acid diversity

between PtIP-65Fa and PtIP-65Fb is indicated with shading.

Figures 8a - 8b show an alignment of the amino acid sequences of PtIP-65Hg and PtIP-65Hh. The amino acid diversity between PtIP-65Hg and PtIP-65Hh is indicated with shading.

Figure 9 shows the phylogeny tree of the PtIP-50 polypeptides: PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, PtIP-50Bd, PtIP-50Fa, PtIP-50Fb, PtIP-50Fd, PtIP-50Fe, PtIP-50Ff, PtIP-50Fg, PtIP-50Fh, PtIP-50Fi, PtIP-50Fj, PtIP-50Fk, PtIP-50Fl, PtIP-50Fm, PtIP-50Fn, PtIP-50Fo, PtIP-50Fp, PtIP-50Fq, PtIP-50Fr, PtIP-50Fs, PtIP-50Ft, PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, and PtIP-50Gd.

Figures 10a - 10o show an alignment of the amino acid sequences of PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, PtIP-50Bd, PtIP-50Fa, PtIP-50Fb, PtIP-50Fd, PtIP-50Fe, PtIP-50Ff, PtIP-50Fg, PtIP-50Fh, PtIP-50Fi, PtIP-50Fj, PtIP-50Fk, PtIP-50Fl, PtIP-50Fm, PtIP-50Fn, PtIP-50Fo, PtIP-50Fp, PtIP-50Fq, PtIP-50Fr, PtIP-50Fs, PtIP-50Ft, PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, and PtIP-50Gd.

Figures 11a - 11f show an alignment of the amino acid sequences of PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, and PtIP-50Bd. The amino acid diversity between PtIP-51Aa and PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, and PtIP-50Bd is indicated with shading.

Figures 12a - 12j show an alignment of the amino acid sequences of PtIP-50Fb, PtIP-50Fe, PtIP-50Fk, PtIP-50Fl, PtIP-50Fn, PtIP-50Fo, PtIP-50Fs, and PtIP-50Ft. The amino acid diversity between PtIP-50Fb and PtIP-50Fe, PtIP-50Fk, PtIP-50Fl, PtIP-50Fn, PtIP-50Fo, PtIP-50Fs, and PtIP-50Ft is indicated with shading.

Figures 13a - 13e show an alignment of the amino acid sequences of PtIP-50Fd, PtIP-50Fg, PtIP-50Fh, PtIP-50Fm, and PtIP-50Fr. The amino acid diversity between PtIP-50Fd and PtIP-50Fg, PtIP-50Fh, PtIP-50Fm, and PtIP-50Fr is indicated with shading.

Figures 14a - 14e show an alignment of the amino acid sequences of PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, and PtIP-50Gd. The amino acid diversity between PtIP-50Ga and PtIP-50Gb, PtIP-50Gc, and PtIP-50Gd is indicated with shading.

Figures 15a - 15e show an alignment of the amino acid sequences of PtIP-50Fa, PtIP-50Ff, PtIP-50Fi, PtIP-50Fj, and PtIP-50Fq. The amino acid diversity between PtIP-50Fa and PtIP-50Ff, PtIP-50Fi, PtIP-50Fj, and PtIP-50Fq is indicated with shading.

Figures 16a - 16u: an alignment of the amino acid sequences of PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, PtIP-50Bd, PtIP-50Fa, PtIP-50Fb, PtIP-50Fd, PtIP-50Fe, PtIP-50Ff, PtIP-50Fg, PtIP-50Fh, PtIP-50Fi, PtIP-50Fj, PtIP-50Fk, PtIP-50Fl, PtIP-50Fm, PtIP-50Fn, PtIP-50Fo, PtIP-50Fp, PtIP-50Fq, PtIP-50Fr, PtIP-50Fs, PtIP-50Ft, PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, and PtIP-50Gd; an alignment of the secondary structure prediction for each of the PtIP-50 polypeptides, by the PSIPRED, top ranked secondary structure prediction method; and the locations of the amino acid sequence motifs, as predicted by MEME motif analysis, relative to PtIP-50Fb. A "H" indicates a predicted helical structure, an "E" indicates a PtIP- beta strand structure, and a "C" indicates a predicted coil structure.

Figures 17a - 17k show an alignment of the amino acid sequences of PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, PtIP-65Ca, PtIP-65Fa, PtIP-65Fb, PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Gd, PtIP-65Ge, PtIP-65Ha, PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, PtIP-65Hf, PtIP-65Hg, PtIP-65Hh, PtIP-65Hj, and PtIP-65Hk; an alignment of the secondary structure prediction for each of the PtIP-65 polypeptides, by the PSIPRED, top ranked secondary structure prediction method; and the locations of the amino acid sequence motifs, as predicted by MEME motif analysis, relative to PtIP-65Gc. A "H" indicates a predicted helical structure, an "E" indicates a PtIP- beta strand structure, and a "C" indicates a predicted coil structure.

Figure 18 shows the effect of the PtIP-50Aa and PtIP-65Aa polypeptides on isolated *Anacarsia gemmatalis* midgut activity. Iscc reflects the transport activity and midgut structural integrity to maintain normal midgut function. The decline in Iscc following the addition of PtIP-50/65 polypeptides reflects the loss of ionic balance in the midgut.

Figure 19 shows a gel image of the specific binding of PtIP-50Aa and PtIP-65Aa to BBMV from *C.includens*.

Figures 20a and 20b show the evaluation of EC₅₀ values from densitometry of gel images for PtIP-50Aa and PtIP-65Aa binding to *C.includens* BBMV. Figure 20a shows the average densitometry values for bound Alexa-PtIP-50Aa in the presence of different concentrations of unlabeled PtIP-50Aa and PtIP-65Aa normalized to the amount bound in the absence of unlabeled PtIP-50Aa and PtIP-65Aa. The solid line reflects the best fit of a square logistic equation to the data. Figure 20b shows the average densitometry values for bound Alexa-PtIP-65Aa in the presence of different concentrations of unlabeled PtIP-50Aa and PtIP-65Aa normalized to the amount bound in the absence of unlabeled PtIP-50Aa and PtIP-65Aa.

DETAILED DESCRIPTION

[0024] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to

which this disclosure belongs unless clearly indicated otherwise.

[0025] The present disclosure is drawn to compositions and methods for controlling pests. The methods involve transforming organisms with nucleic acid sequences encoding PtIP-50 polypeptides and PtIP-65 polypeptides. In particular, the nucleic acid sequences of the embodiments are useful for preparing plants and microorganisms that possess pesticidal activity. Thus, transformed bacteria, plants, plant cells, plant tissues and seeds are provided. The compositions are pesticidal nucleic acids and proteins of fern species. The nucleic acid sequences find use in the construction of expression vectors for subsequent transformation into organisms of interest, as probes for the isolation of other homologous (or partially homologous) genes, and for the generation of altered PtIP-50 polypeptides and PtIP-65 polypeptides by methods known in the art, such as site directed mutagenesis, domain swapping or DNA shuffling. The PtIP-50 polypeptides and PtIP-65 polypeptides find use in controlling or killing Lepidopteran, Coleopteran, Dipteran, fungal, Hemipteran and nematode pest populations and for producing compositions with pesticidal activity. Insect pests of interest include, but are not limited to, Lepidoptera species including but not limited to: Corn Earworm, (CEW) (*Helicoverpa zea*), European Corn Borer (ECB) (*Ostrinia nubilalis*), diamond-back moth, e.g., *Helicoverpa zea* Boddie; soybean looper, e.g., *Pseudoplusia includens* Walker; and velvet bean caterpillar e.g., *Anticarsia gemmatilis* Hübner and Coleoptera species including but not limited to Western corn rootworm (*Diabrotica virgifera*) - WCRW, Southern corn rootworm (*Diabrotica undecimpunctata howardi*) - SCRW, and Northern corn rootworm (*Diabrotica barberi*) - NCRW.

[0026] By "pesticidal toxin" or "pesticidal protein" is used herein to refer to a toxin that has toxic activity against one or more pests, including, but not limited to, members of the Lepidoptera, Diptera, Hemiptera and Coleoptera orders or the Nematoda phylum or a protein that has homology to such a protein. Pesticidal proteins have been isolated from organisms including, for example, *Bacillus* sp., *Pseudomonas* sp., *Photorhabdus* sp., *Xenorhabdus* sp., *Clostridium bifermentans* and *Paenibacillus popilliae*. Pesticidal proteins include but are not limited to: insecticidal proteins from *Pseudomonas* sp. such as PSEEN3174 (Monalysin; (2011) PLoS Pathogens 7:1-13); from *Pseudomonas protegens* strain CHAO and Pf-5 (previously *fluorescens*) (Pechy-Tarr, (2008) Environmental Microbiology 10:2368-2386; GenBank Accession No. EU400157); from *Pseudomonas Taiwanensis* (Liu, et al., (2010) J. Agric. Food Chem., 58:12343-12349) and from *Pseudomonas pseudoalcaligenes* (Zhang, et al., (2009) Annals of Microbiology 59:45-50 and Li, et al., (2007) Plant Cell Tiss. Organ Cult. 89:159-168); insecticidal proteins from *Photorhabdus* sp. and *Xenorhabdus* sp. (Hinchliffe, et al., (2010) The Open Toxicology Journal, 3:101-118 and Morgan, et al., (2001) Applied and Envir. Micro. 67:2062-2069); US Patent Number 6,048,838, and US Patent Number 6,379,946; a PIP-1 polypeptide of US Serial Number 13/792861; an AfIP-1A and/or AfIP-1B polypeptide of US Serial Number 13/800233; a PHI-4 polypeptide of US Serial Number 13/839702; and δ -endotoxins including, but not limited to, the Cry1, Cry2, Cry3, Cry4, Cry5, Cry6, Cry7, Cry8, Cry9, Cry10, Cry11, Cry12, Cry13, Cry14, Cry15, Cry16, Cry17, Cry18, Cry19, Cry20, Cry21, Cry22, Cry23, Cry24, Cry25, Cry26, Cry27, Cry 28, Cry 29, Cry 30, Cry31, Cry32, Cry33, Cry34, Cry35, Cry36, Cry37, Cry38, Cry39, Cry40, Cry41, Cry42, Cry43, Cry44, Cry45, Cry 46, Cry47, Cry49, Cry 51 and Cry55 classes of δ -endotoxin genes and the *B. thuringiensis* cytolytic cyt1 and cyt2 genes. Members of these classes of *B. thuringiensis* insecticidal proteins include, but are not limited to Cry1Aa1 (Accession # AAA22353); Cry1Aa2 (Accession # Accession # AAA22552); Cry1Aa3 (Accession # BAA00257); Cry1Aa4 (Accession # CAA31886); Cry1Aa5 (Accession # BAA04468); Cry1Aa6 (Accession # AAA86265); Cry1Aa7 (Accession # AAD46139); Cry1Aa8 (Accession # 126149); Cry1Aa9 (Accession # BAA77213); Cry1Aa10 (Accession # AAD55382); Cry1Aa11 (Accession # CAA70856); Cry1Aa12 (Accession # AAP80146); Cry1Aa13 (Accession # AAM44305); Cry1Aa14 (Accession # AAP40639); Cry1Aa15 (Accession # AAY66993); Cry1Aa16 (Accession # HQ439776); Cry1Aa17 (Accession # HQ439788); Cry1Aa18 (Accession # HQ439790); Cry1Aa19 (Accession # HQ685121); Cry1Aa20 (Accession # JF340156); Cry1Aa21 (Accession # JN651496); Cry1Aa22 (Accession # KC158223); Cry1Ab1 (Accession # AAA22330); Cry1Ab2 (Accession # AAA22613); Cry1Ab3 (Accession # AAA22561); Cry1Ab4 (Accession # BAA00071); Cry1Ab5 (Accession # CAA28405); Cry1Ab6 (Accession # AAA22420); Cry1Ab7 (Accession # CAA31620); Cry1Ab8 (Accession # AAA22551); Cry1Ab9 (Accession # CAA38701); Cry1Ab10 (Accession # A29125); Cry1Ab11 (Accession # 112419); Cry1Ab12 (Accession # AAC64003); Cry1Ab13 (Accession # AAN76494); Cry1Ab14 (Accession # AAG16877); Cry1Ab15 (Accession # AAO13302); Cry1Ab16 (Accession # AAK55546); Cry1Ab17 (Accession # AAT46415); Cry1Ab18 (Accession # AAQ88259); Cry1Ab19 (Accession # AAW31761); Cry1Ab20 (Accession # ABB72460); Cry1Ab21 (Accession # ABS18384); Cry1Ab22 (Accession # ABW87320); Cry1Ab23 (Accession # HQ439777); Cry1Ab24 (Accession # HQ439778); Cry1Ab25 (Accession # HQ685122); Cry1Ab26 (Accession # HQ847729); Cry1Ab27 (Accession # JN135249); Cry1Ab28 (Accession # JN135250); Cry1Ab29 (Accession # JN135251); Cry1Ab30 (Accession # JN135252); Cry1Ab31 (Accession # JN135253); Cry1Ab32 (Accession # JN135254); Cry1Ab33 (Accession # AAS93798); Cry1Ab34 (Accession # KC156668); Cry1Ab-like (Accession # AAK14336); Cry1Ab-like (Accession # AAK14337); Cry1Ab-like (Accession # AAK14338); Cry1Ab-like (Accession # ABG88858); Cry1Ac1 (Accession # AAA22331); Cry1Ac2 (Accession # AAA22338); Cry1Ac3 (Accession # CAA38098); Cry1Ac4 (Accession # AAA73077); Cry1Ac5 (Accession # AAA22339); Cry1Ac6 (Accession # AAA86266); Cry1Ac7 (Accession # AAB46989); Cry1Ac8 (Accession # AAC44841); Cry1Ac9 (Accession # AAB49768); Cry1Ac10 (Accession # CAA05505); Cry1Ac11 (Accession # CAA10270); Cry1Ac12 (Accession # 112418); Cry1Ac13 (Accession # AAD38701); Cry1Ac14 (Accession # AAQ06607); Cry1Ac15 (Accession # AAN07788); Cry1Ac16 (Accession #

EP 3 102 684 B1

AAU87037); Cry1Ac17 (Accession # AAX18704); Cry1Ac18 (Accession # AAY88347); Cry1Ac19 (Accession # ABD37053); Cry1Ac20 (Accession # ABB89046); Cry1Ac21 (Accession # AAY66992); Cry1Ac22 (Accession # ABZ01836); Cry1Ac23 (Accession # CAQ30431); Cry1Ac24 (Accession # ABL01535); Cry1Ac25 (Accession # FJ513324); Cry1Ac26 (Accession # FJ617446); Cry1Ac27 (Accession # FJ617447); Cry1Ac28 (Accession # ACM90319);
5 Cry1Ac29 (Accession # DQ438941); Cry1Ac30 (Accession # GQ227507); Cry1Ac31 (Accession # GU446674); Cry1Ac32 (Accession # HM061081); Cry1Ac33 (Accession # GQ866913); Cry1Ac34 (Accession # HQ230364); Cry1Ac35 (Accession # JF340157); Cry1Ac36 (Accession # JN387137); Cry1Ac37 (Accession # JQ317685); Cry1Ad1 (Accession # AAA22340); Cry1Ad2 (Accession # CAA01880); Cry1Ae1 (Accession # AAA22410); Cry1Af1 (Accession # AAB82749); Cry1Ag1 (Accession # AAD46137); Cry1Ah1 (Accession # AAQ14326); Cry1Ah2 (Accession # ABB76664); Cry1Ah3 (Accession # HQ439779); Cry1Ai1 (Accession # AAO39719); Cry1Ai2 (Accession # HQ439780); Cry1A-like (Accession # AAK14339); Cry1Ba1 (Accession # CAA29898); Cry1Ba2 (Accession # CAA65003); Cry1Ba3 (Accession # AAK63251); Cry1Ba4 (Accession # AAK51084); Cry1Ba5 (Accession # ABO20894); Cry1Ba6 (Accession # ABL60921); Cry1Ba7 (Accession # HQ439781); Cry1Bb1 (Accession # AAA22344); Cry1Bb2 (Accession # HQ439782); Cry1Bc1 (Accession # CAA86568); Cry1Bd1 (Accession # AAD10292); Cry1Bd2 (Accession # AAM93496); Cry1Be1 (Accession # AAC32850); Cry1Be2 (Accession # AAQ52387); Cry1Be3 (Accession # ACV96720); Cry1Be4 (Accession # HM070026); Cry1Bf1 (Accession # CAC50778); Cry1Bf2 (Accession # AAQ52380); Cry1Bg1 (Accession # AAO39720); Cry1Bh1 (Accession # HQ589331); Cry1Bi1 (Accession # KC156700); Cry1Ca1 (Accession # CAA30396); Cry1Ca2 (Accession # CAA31951); Cry1Ca3 (Accession # AAA22343); Cry1Ca4 (Accession # CAA01886); Cry1Ca5 (Accession # CAA65457); Cry1Ca6 [1] (Accession # AAF37224); Cry1Ca7 (Accession # AAG50438); Cry1Ca8 (Accession # AAM00264); Cry1Ca9 (Accession # AAL79362); Cry1Ca10 (Accession # AAN16462); Cry1Ca11 (Accession # AAX53094); Cry1Ca12 (Accession # HM070027); Cry1Ca13 (Accession # HQ412621); Cry1Ca14 (Accession # JN651493); Cry1Cb1 (Accession # M97880); Cry1Cb2 (Accession # AAG35409); Cry1Cb3 (Accession # ACD50894); Cry1Cb-like (Accession # AAX63901); Cry1Da1 (Accession # CAA38099); Cry1Da2 (Accession # 176415); Cry1Da3 (Accession # HQ439784); Cry1Db1 (Accession # CAA80234); Cry1Db2 (Accession # AAK48937); Cry1Dc1 (Accession # ABK35074); Cry1Ea1 (Accession # CAA37933); Cry1Ea2 (Accession # CAA39609); Cry1Ea3 (Accession # AAA22345); Cry1Ea4 (Accession # AAD04732); Cry1Ea5 (Accession # A15535); Cry1Ea6 (Accession # AAL50330); Cry1Ea7 (Accession # AAW72936); Cry1Ea8 (Accession # ABX11258); Cry1Ea9 (Accession # HQ439785); Cry1Ea10 (Accession # ADR00398); Cry1Ea11 (Accession # JQ652456); Cry1Eb1 (Accession # AAA22346); Cry1Fa1 (Accession # AAA22348); Cry1Fa2 (Accession # AAA22347); Cry1Fa3 (Accession # HM070028); Cry1Fa4 (Accession # HM439638);
30 Cry1Fb1 (Accession # CAA80235); Cry1Fb2 (Accession # BAA25298); Cry1Fb3 (Accession # AAF21767); Cry1Fb4 (Accession # AAC10641); Cry1Fb5 (Accession # AAO13295); Cry1Fb6 (Accession # ACD50892); Cry1Fb7 (Accession # ACD50893); Cry1Ga1 (Accession # CAA80233); Cry1Ga2 (Accession # CAA70506); Cry1Gb1 (Accession # AAD10291); Cry1Gb2 (Accession # AAO13756); Cry1Gc1 (Accession # AAQ52381); Cry1Ha1 (Accession # CAA80236); Cry1Hb1 (Accession # AAA79694); Cry1Hb2 (Accession # HQ439786); Cry1H-like (Accession # AAF01213); Cry1Ia1 (Accession # CAA44633); Cry1Ia2 (Accession # AAA22354); Cry1Ia3 (Accession # AAC36999); Cry1Ia4 (Accession # AAB00958); Cry1Ia5 (Accession # CAA70124); Cry1Ia6 (Accession # AAC26910); Cry1Ia7 (Accession # AAM73516); Cry1Ia8 (Accession # AAK66742); Cry1Ia9 (Accession # AAQ08616); Cry1Ia10 (Accession # AAP86782); Cry1Ia11 (Accession # CAC85964); Cry1Ia12 (Accession # AAV53390); Cry1Ia13 (Accession # ABF83202); Cry1Ia14 (Accession # ACG63871); Cry1Ia15 (Accession # FJ617445); Cry1Ia16 (Accession # FJ617448); Cry1Ia17 (Accession # GU989199);
40 Cry1Ia18 (Accession # ADK23801); Cry1Ia19 (Accession # HQ439787); Cry1Ia20 (Accession # JQ228426); Cry1Ia21 (Accession # JQ228424); Cry1Ia22 (Accession # JQ228427); Cry1Ia23 (Accession # JQ228428); Cry1Ia24 (Accession # JQ228429); Cry1Ia25 (Accession # JQ228430); Cry1Ia26 (Accession # JQ228431); Cry1Ia27 (Accession # JQ228432); Cry1Ia28 (Accession # JQ228433); Cry1Ia29 (Accession # JQ228434); Cry1Ia30 (Accession # JQ317686); Cry1Ia31 (Accession # JX944038); Cry1Ia32 (Accession # JX944039); Cry1Ia33 (Accession # JX944040); Cry1Ib1 (Accession # AAA82114); Cry1Ib2 (Accession # ABW88019); Cry1Ib3 (Accession # ACD75515); Cry1Ib4 (Accession # HM051227); Cry1Ib5 (Accession # HM070028); Cry1Ib6 (Accession # ADK38579); Cry1Ib7 (Accession # JN571740); Cry1Ib8 (Accession # JN675714); Cry1Ib9 (Accession # JN675715); Cry1Ib10 (Accession # JN675716); Cry1Ib11 (Accession # JQ228423); Cry1Ic1 (Accession # AAC62933); Cry1Ic2 (Accession # AAET1691); Cry1Id1 (Accession # AAD44366); Cry1Id2 (Accession # JQ228422); Cry1Ie1 (Accession # AAG43526); Cry1Ie2 (Accession # HM439636); Cry1Ie3 (Accession # KC156647); Cry1Ie4 (Accession # KC156681); Cry1If1 (Accession # AAQ52382); Cry1Ig1 (Accession # KC156701); Cry1I-like (Accession # AAC31094); Cry1I-like (Accession # ABG88859); Cry1Ja1 (Accession # AAA22341); Cry1Ja2 (Accession # HM070030); Cry1Ja3 (Accession # JQ228425); Cry1Jb1 (Accession # AAA98959); Cry1Jc1 (Accession # AAC31092); Cry1Jc2 (Accession # AAQ52372); Cry1Jd1 (Accession # CAC50779); Cry1Ka1 (Accession # AAB00376); Cry1Ka2 (Accession # HQ439783); Cry1La1 (Accession # AAS60191); Cry1La2 (Accession # HM070031);
55 Cry1Ma1 (Accession # FJ884067); Cry1Ma2 (Accession # KC156659); Cry1Na1 (Accession # KC156648); Cry1Nb1 (Accession # KC156678); Cry1-like (Accession # AAC31091); Cry2Aa1 (Accession # AAA22335); Cry2Aa2 (Accession # AAA83516); Cry2Aa3 (Accession # D86064); Cry2Aa4 (Accession # AAC04867); Cry2Aa5 (Accession # CAA10671); Cry2Aa6 (Accession # CAA10672); Cry2Aa7 (Accession # CAA10670); Cry2Aa8 (Accession # AAO13734); Cry2Aa9

EP 3 102 684 B1

(Accession # AAO13750); Cry2Aa10 (Accession # AAQ04263); Cry2Aa11 (Accession # AAQ52384); Cry2Aa12 (Accession # ABI83671); Cry2Aa13 (Accession # ABL01536); Cry2Aa14 (Accession # ACF04939); Cry2Aa15 (Accession # JN426947); Cry2Ab1 (Accession # AAA22342); Cry2Ab2 (Accession # CAA39075); Cry2Ab3 (Accession # AAG36762); Cry2Ab4 (Accession # AAO13296); Cry2Ab5 (Accession # AAQ04609); Cry2Ab6 (Accession # AAP59457); Cry2Ab7 (Accession # AAZ66347); Cry2Ab8 (Accession # ABC95996); Cry2Ab9 (Accession # ABC74968); Cry2Ab10 (Accession # EF157306); Cry2Ab11 (Accession # CAM84575); Cry2Ab12 (Accession # ABM21764); Cry2Ab13 (Accession # ACG76120); Cry2Ab14 (Accession # ACG76121); Cry2Ab15 (Accession # HM037126); Cry2Ab16 (Accession # GQ866914); Cry2Ab17 (Accession # HQ439789); Cry2Ab18 (Accession # JN135255); Cry2Ab19 (Accession # JN135256); Cry2Ab20 (Accession # JN135257); Cry2Ab21 (Accession # JN135258); Cry2Ab22 (Accession # JN135259); Cry2Ab23 (Accession # JN135260); Cry2Ab24 (Accession # JN135261); Cry2Ab25 (Accession # JN415485); Cry2Ab26 (Accession # JN426946); Cry2Ab27 (Accession # JN415764); Cry2Ab28 (Accession # JN651494); Cry2Ac1 (Accession # CAA40536); Cry2Ac2 (Accession # AAG35410); Cry2Ac3 (Accession # AAQ52385); Cry2Ac4 (Accession # ABC95997); Cry2Ac5 (Accession # ABC74969); Cry2Ac6 (Accession # ABC74793); Cry2Ac7 (Accession # CAL18690); Cry2Ac8 (Accession # CAM09325); Cry2Ac9 (Accession # CAM09326); Cry2Ac10 (Accession # ABN15104); Cry2Ac11 (Accession # CAM83895); Cry2Ac12 (Accession # CAM83896); Cry2Ad1 (Accession # AAF09583); Cry2Ad2 (Accession # ABC86927); Cry2Ad3 (Accession # CAK29504); Cry2Ad4 (Accession # CAM32331); Cry2Ad5 (Accession # CAO78739); Cry2Ae1 (Accession # AAQ52362); Cry2Af1 (Accession # ABO30519); Cry2Af2 (Accession # GQ866915); Cry2Ag1 (Accession # ACH91610); Cry2Ah1 (Accession # EU939453); Cry2Ah2 (Accession # ACL80665); Cry2Ah3 (Accession # GU073380); Cry2Ah4 (Accession # KC156702); Cry2Ai1 (Accession # FJ788388); Cry2Aj (Accession #); Cry2Ak1 (Accession # KC156660); Cry2Ba1 (Accession # KC156658); Cry3Aa1 (Accession # AAA22336); Cry3Aa2 (Accession # AAA22541); Cry3Aa3 (Accession # CAA68482); Cry3Aa4 (Accession # AAA22542); Cry3Aa5 (Accession # AAA50255); Cry3Aa6 (Accession # AAC43266); Cry3Aa7 (Accession # CAB41411); Cry3Aa8 (Accession # AAS79487); Cry3Aa9 (Accession # AAW05659); Cry3Aa10 (Accession # AAU29411); Cry3Aa11 (Accession # AAW82872); Cry3Aa12 (Accession # ABY49136); Cry3Ba1 (Accession # CAA34983); Cry3Ba2 (Accession # CAA00645); Cry3Ba3 (Accession # JQ397327); Cry3Bb1 (Accession # AAA22334); Cry3Bb2 (Accession # AAA74198); Cry3Bb3 (Accession # 115475); Cry3Ca1 (Accession # CAA42469); Cry4Aa1 (Accession # CAA68485); Cry4Aa2 (Accession # BAA00179); Cry4Aa3 (Accession # CAD30148); Cry4Aa4 (Accession # AFB18317); Cry4A-like (Accession # AAY96321); Cry4Ba1 (Accession # CAA30312); Cry4Ba2 (Accession # CAA30114); Cry4Ba3 (Accession # AAA22337); Cry4Ba4 (Accession # BAA00178); Cry4Ba5 (Accession # CAD30095); Cry4Ba-like (Accession # ABC47686); Cry4Ca1 (Accession # EU646202); Cry4Cb1 (Accession # FJ403208); Cry4Cb2 (Accession # FJ597622); Cry4Cc1 (Accession # FJ403207); Cry5Aa1 (Accession # AAA67694); Cry5Ab1 (Accession # AAA67693); Cry5Ac1 (Accession # 134543); Cry5Ad1 (Accession # ABQ82087); Cry5Ba1 (Accession # AAA68598); Cry5Ba2 (Accession # ABW88931); Cry5Ba3 (Accession # AFJ04417); Cry5Ca1 (Accession # HM461869); Cry5Ca2 (Accession # ZP_04123426); Cry5Da1 (Accession # HM461870); Cry5Da2 (Accession # ZP_04123980); Cry5Ea1 (Accession # HM485580); Cry5Ea2 (Accession # ZP_04124038); Cry6Aa1 (Accession # AAA22357); Cry6Aa2 (Accession # AAM46849); Cry6Aa3 (Accession # ABH03377); Cry6Ba1 (Accession # AAA22358); Cry7Aa1 (Accession # AAA22351); Cry7Ab1 (Accession # AAA21120); Cry7Ab2 (Accession # AAA21121); Cry7Ab3 (Accession # ABX24522); Cry7Ab4 (Accession # EU380678); Cry7Ab5 (Accession # ABX79555); Cry7Ab6 (Accession # ACI44005); Cry7Ab7 (Accession # ADB89216); Cry7Ab8 (Accession # GU145299); Cry7Ab9 (Accession # ADD92572); Cry7Ba1 (Accession # ABB70817); Cry7Bb1 (Accession # KC156653); Cry7Ca1 (Accession # ABR67863); Cry7Cb1 (Accession # KC156698); Cry7Da1 (Accession # ACQ99547); Cry7Da2 (Accession # HM572236); Cry7Da3 (Accession # KC156679); Cry7Ea1 (Accession # HM035086); Cry7Ea2 (Accession # HM132124); Cry7Ea3 (Accession # EEM19403); Cry7Fa1 (Accession # HM035088); Cry7Fa2 (Accession # EEM19090); Cry7Fb1 (Accession # HM572235); Cry7Fb2 (Accession # KC156682); Cry7Ga1 (Accession # HM572237); Cry7Ga2 (Accession # KC156669); Cry7Gb1 (Accession # KC156650); Cry7Gc1 (Accession # KC156654); Cry7Gd1 (Accession # KC156697); Cry7Ha1 (Accession # KC156651); Cry7Ia1 (Accession # KC156665); Cry7Ja1 (Accession # KC156671); Cry7Ka1 (Accession # KC156680); Cry7Kb1 (Accession # BAM99306); Cry7La1 (Accession # BAM99307); Cry8Aa1 (Accession # AAA21117); Cry8Ab1 (Accession # EU044830); Cry8Ac1 (Accession # KC156662); Cry8Ad1 (Accession # KC156684); Cry8Ba1 (Accession # AAA21118); Cry8Bb1 (Accession # CAD57542); Cry8Bc1 (Accession # CAD57543); Cry8Ca1 (Accession # AAA21119); Cry8Ca2 (Accession # AAR98783); Cry8Ca3 (Accession # EU625349); Cry8Ca4 (Accession # ADB54826); Cry8Da1 (Accession # BAC07226); Cry8Da2 (Accession # BD133574); Cry8Da3 (Accession # BD133575); Cry8Db1 (Accession # BAF93483); Cry8Ea1 (Accession # AAQ73470); Cry8Ea2 (Accession # EU047597); Cry8Ea3 (Accession # KC855216); Cry8Fa1 (Accession # AAT48690); Cry8Fa2 (Accession # HQ174208); Cry8Fa3 (Accession # AFH78109); Cry8Ga1 (Accession # AAT46073); Cry8Ga2 (Accession # ABC42043); Cry8Ga3 (Accession # FJ198072); Cry8Ha1 (Accession # AAW81032); Cry8Ia1 (Accession # EU381044); Cry8Ia2 (Accession # GU073381); Cry8Ia3 (Accession # HM044664); Cry8Ia4 (Accession # KC156674); Cry8Ib1 (Accession # GU325772); Cry8Ib2 (Accession # KC156677); Cry8Ja1 (Accession # EU625348); Cry8Ka1 (Accession # FJ422558); Cry8Ka2 (Accession # ACN87262); Cry8Kb1 (Accession # HM123758); Cry8Kb2 (Accession # KC156675); Cry8La1 (Accession # GU325771); Cry8Ma1 (Accession # HM044665);

EP 3 102 684 B1

Cry8Ma2 (Accession # EEM86551); Cry8Ma3 (Accession # HM210574); Cry8Na1 (Accession # HM640939); Cry8Pa1 (Accession # HQ388415); Cry8Qa1 (Accession # HQ441166); Cry8Qa2 (Accession # KC152468); Cry8Ra1 (Accession # AFP87548); Cry8Sa1 (Accession # JQ740599); Cry8Ta1 (Accession # KC156673); Cry8-like (Accession # FJ770571); Cry8-like (Accession # ABS53003); Cry9Aa1 (Accession # CAA41122); Cry9Aa2 (Accession # CAA41425); Cry9Aa3 (Accession # GQ249293); Cry9Aa4 (Accession # GQ249294); Cry9Aa5 (Accession # JX174110); Cry9Aa like (Accession # AAQ52376); Cry9Ba1 (Accession # CAA52927); Cry9Ba2 (Accession # GU299522); Cry9Bb1 (Accession # AAV28716); Cry9Ca1 (Accession # CAA85764); Cry9Ca2 (Accession # AAQ52375); Cry9Da1 (Accession # BAA19948); Cry9Da2 (Accession # AAB97923); Cry9Da3 (Accession # GQ249293); Cry9Da4 (Accession # GQ249297); Cry9Db1 (Accession # AAX78439); Cry9Dc1 (Accession # KC156683); Cry9Ea1 (Accession # BAA34908); Cry9Ea2 (Accession # AAO12908); Cry9Ea3 (Accession # ABM21765); Cry9Ea4 (Accession # ACE88267); Cry9Ea5 (Accession # ACF04743); Cry9Ea6 (Accession # ACG63872); Cry9Ea7 (Accession # FJ380927); Cry9Ea8 (Accession # GQ249292); Cry9Ea9 (Accession # JN651495); Cry9Eb1 (Accession # CAC50780); Cry9Eb2 (Accession # GQ249298); Cry9Eb3 (Accession # KC156646); Cry9Ec1 (Accession # AAC63366); Cry9Ed1 (Accession # AAX78440); Cry9Ee1 (Accession # GQ249296); Cry9Ee2 (Accession # KC156664); Cry9Fa1 (Accession # KC156692); Cry9Ga1 (Accession # KC156699); Cry9-like (Accession # AAC63366); Cry10Aa1 (Accession # AAA22614); Cry10Aa2 (Accession # E00614); Cry10Aa3 (Accession # CAD30098); Cry10Aa4 (Accession # AFB18318); Cry10A-like (Accession # DQ167578); Cry11Aa1 (Accession # AAA22352); Cry11Aa2 (Accession # AAA22611); Cry11Aa3 (Accession # CAD30081); Cry11Aa4 (Accession # AFB18319); Cry11Aa-like (Accession # DQ166531); Cry11Ba1 (Accession # CAA60504); Cry11Bb1 (Accession # AAC97162); Cry11Bb2 (Accession # HM068615); Cry12Aa1 (Accession # AAA22355); Cry13Aa1 (Accession # AAA22356); Cry14Aa1 (Accession # AAA21516); Cry14Ab1 (Accession # KC156652); Cry15Aa1 (Accession # AAA22333); Cry16Aa1 (Accession # CAA63860); Cry17Aa1 (Accession # CAA67841); Cry18Aa1 (Accession # CAA67506); Cry18Ba1 (Accession # AAF89667); Cry18Ca1 (Accession # AAF89668); Cry19Aa1 (Accession # CAA68875); Cry19Ba1 (Accession # BAA32397); Cry19Ca1 (Accession # AFM37572); Cry20Aa1 (Accession # AAB93476); Cry20Ba1 (Accession # ACS93601); Cry20Ba2 (Accession # KC156694); Cry20-like (Accession # GQ144333); Cry21Aa1 (Accession # I32932); Cry21Aa2 (Accession # I66477); Cry21Ba1 (Accession # BAC06484); Cry21Ca1 (Accession # JF521577); Cry21Ca2 (Accession # KC156687); Cry21Da1 (Accession # JF521578); Cry22Aa1 (Accession # I34547); Cry22Aa2 (Accession # CAD43579); Cry22Aa3 (Accession # ACD93211); Cry22Ab1 (Accession # AAK50456); Cry22Ab2 (Accession # CAD43577); Cry22Ba1 (Accession # CAD43578); Cry22Bb1 (Accession # KC156672); Cry23Aa1 (Accession # AAF76375); Cry24Aa1 (Accession # AAC61891); Cry24Ba1 (Accession # BAD32657); Cry24Ca1 (Accession # CAJ43600); Cry25Aa1 (Accession # AAC61892); Cry26Aa1 (Accession # AAD25075); Cry27Aa1 (Accession # BAA82796); Cry28Aa1 (Accession # AAD24189); Cry28Aa2 (Accession # AAG00235); Cry29Aa1 (Accession # CAC80985); Cry30Aa1 (Accession # CAC80986); Cry30Ba1 (Accession # BAD00052); Cry30Ca1 (Accession # BAD67157); Cry30Ca2 (Accession # ACU24781); Cry30Da1 (Accession # EF095955); Cry30Db1 (Accession # BAE80088); Cry30Ea1 (Accession # ACC95445); Cry30Ea2 (Accession # FJ499389); Cry30Fa1 (Accession # ACI22625); Cry30Ga1 (Accession # ACG60020); Cry30Ga2 (Accession # HQ638217); Cry31Aa1 (Accession # BAB11757); Cry31Aa2 (Accession # AAL87458); Cry31Aa3 (Accession # BAE79808); Cry31Aa4 (Accession # BAF32571); Cry31Aa5 (Accession # BAF32572); Cry31Aa6 (Accession # BAI44026); Cry31Ab1 (Accession # BAE79809); Cry31Ab2 (Accession # BAF32570); Cry31Ac1 (Accession # BAF34368); Cry31Ac2 (Accession # AB731600); Cry31Ad1 (Accession # BAI44022); Cry32Aa1 (Accession # AAG36711); Cry32Aa2 (Accession # GU063849); Cry32Ab1 (Accession # GU063850); Cry32Ba1 (Accession # BAB78601); Cry32Ca1 (Accession # BAB78602); Cry32Cb1 (Accession # KC156708); Cry32Da1 (Accession # BAB78603); Cry32Ea1 (Accession # GU324274); Cry32Ea2 (Accession # KC156686); Cry32Eb1 (Accession # KC156663); Cry32Fa1 (Accession # KC156656); Cry32Ga1 (Accession # KC156657); Cry32Ha1 (Accession # KC156661); Cry32Hb1 (Accession # KC156666); Cry32Ia1 (Accession # KC156667); Cry32Ja1 (Accession # KC156685); Cry32Ka1 (Accession # KC156688); Cry32La1 (Accession # KC156689); Cry32Ma1 (Accession # KC156690); Cry32Mb1 (Accession # KC156704); Cry32Na1 (Accession # KC156691); Cry32Oa1 (Accession # KC156703); Cry32Pa1 (Accession # KC156705); Cry32Qa1 (Accession # KC156706); Cry32Ra1 (Accession # KC156707); Cry32Sa1 (Accession # KC156709); Cry32Ta1 (Accession # KC156710); Cry32Ua1 (Accession # KC156655); Cry33Aa1 (Accession # AAL26871); Cry34Aa1 (Accession # AAG50341); Cry34Aa2 (Accession # AAK64560); Cry34Aa3 (Accession # AAT29032); Cry34Aa4 (Accession # AAT29030); Cry34Ab1 (Accession # AAG41671); Cry34Ac1 (Accession # AAG50118); Cry34Ac2 (Accession # AAK64562); Cry34Ac3 (Accession # AAT29029); Cry34Ba1 (Accession # AAK64565); Cry34Ba2 (Accession # AAT29033); Cry34Ba3 (Accession # AAT29031); Cry35Aa1 (Accession # AAG50342); Cry35Aa2 (Accession # AAK64561); Cry35Aa3 (Accession # AAT29028); Cry35Aa4 (Accession # AAT29025); Cry35Ab1 (Accession # AAG41672); Cry35Ab2 (Accession # AAK64563); Cry35Ab3 (Accession # AY536891); Cry35Ac1 (Accession # AAG50117); Cry35Ba1 (Accession # AAK64566); Cry35Ba2 (Accession # AAT29027); Cry35Ba3 (Accession # AAT29026); Cry36Aa1 (Accession # AAK64558); Cry37Aa1 (Accession # AAF76376); Cry38Aa1 (Accession # AAK64559); Cry39Aa1 (Accession # BAB72016); Cry40Aa1 (Accession # BAB72018); Cry40Ba1 (Accession # BAC77648); Cry40Ca1 (Accession #

EP 3 102 684 B1

EU381045); Cry40Da1 (Accession # ACF15199); Cry41Aa1 (Accession # BAD35157); Cry41Ab1 (Accession # BAD35163); Cry41Ba1 (Accession # HM461871); Cry41Ba2 (Accession # ZP_04099652); Cry42Aa1 (Accession # BAD35166); Cry43Aa1 (Accession # BAD15301); Cry43Aa2 (Accession # BAD95474); Cry43Ba1 (Accession # BAD15303); Cry43Ca1 (Accession # KC156676); Cry43Cb1 (Accession # KC156695); Cry43Cc1 (Accession # KC156696); Cry43-like (Accession # BAD15305); Cry44Aa (Accession # BAD08532); Cry45Aa (Accession # BAD22577); Cry46Aa (Accession # BAC79010); Cry46Aa2 (Accession # BAG68906); Cry46Ab (Accession # BAD35170); Cry47Aa (Accession # AAY24695); Cry48Aa (Accession # CAJ18351); Cry48Aa2 (Accession # CAJ86545); Cry48Aa3 (Accession # CAJ86546); Cry48Ab (Accession # CAJ86548); Cry48Ab2 (Accession # CAJ86549); Cry49Aa (Accession # CAH56541); Cry49Aa2 (Accession # CAJ86541); Cry49Aa3 (Accession # CAJ86543); Cry49Aa4 (Accession # CAJ86544); Cry49Ab1 (Accession # CAJ86542); Cry50Aa1 (Accession # BAE86999); Cry50Ba1 (Accession # GU446675); Cry50Ba2 (Accession # GU446676); Cry51Aa1 (Accession # ABI14444); Cry51Aa2 (Accession # GU570697); Cry52Aa1 (Accession # EF613489); Cry52Ba1 (Accession # FJ361760); Cry53Aa1 (Accession # EF633476); Cry53Ab1 (Accession # FJ361759); Cry54Aa1 (Accession # ACA52194); Cry54Aa2 (Accession # GQ140349); Cry54Ba1 (Accession # GU446677); Cry55Aa1 (Accession # ABW88932); Cry54Ab1 (Accession # JQ916908); Cry55Aa2 (Accession # AAE33526); Cry56Aa1 (Accession # ACU57499); Cry56Aa2 (Accession # GQ483512); Cry56Aa3 (Accession # JX025567); Cry57Aa1 (Accession # ANC87261); Cry58Aa1 (Accession # ANC87260); Cry59Ba1 (Accession # JN790647); Cry59Aa1 (Accession # ACR43758); Cry60Aa1 (Accession # ACU24782); Cry60Aa2 (Accession # EAO57254); Cry60Aa3 (Accession # EEM99278); Cry60Ba1 (Accession # GU810818); Cry60Ba2 (Accession # EAO57253); Cry60Ba3 (Accession # EEM99279); Cry61Aa1 (Accession # HM035087); Cry61Aa2 (Accession # HM132125); Cry61Aa3 (Accession # EEM19308); Cry62Aa1 (Accession # HM054509); Cry63Aa1 (Accession # BAI44028); Cry64Aa1 (Accession # BAJ05397); Cry65Aa1 (Accession # HM461868); Cry65Aa2 (Accession # ZP_04123838); Cry66Aa1 (Accession # HM485581); Cry66Aa2 (Accession # ZP_04099945); Cry67Aa1 (Accession # HM485582); Cry67Aa2 (Accession # ZP_04148882); Cry68Aa1 (Accession # HQ113114); Cry69Aa1 (Accession # HQ401006); Cry69Aa2 (Accession # JQ821388); Cry69Ab1 (Accession # JN209957); Cry70Aa1 (Accession # JN646781); Cry70Ba1 (Accession # ADO51070); Cry70Bb1 (Accession # EEL67276); Cry71Aa1 (Accession # JX025568); Cry72Aa1 (Accession # JX025569); Cyt1Aa (GenBank Accession Number X03182); Cyt1Ab (GenBank Accession Number X98793); Cyt1B (GenBank Accession Number U37196); Cyt2A (GenBank Accession Number Z14147); and Cyt2B (GenBank Accession Number U52043).

[0027] Examples of δ -endotoxins also include but are not limited to Cry1A proteins of US Patent Numbers 5,880,275 and 7,858,849; a DIG-3 or DIG-11 toxin (N-terminal deletion of α -helix 1 and/or α -helix 2 variants of cry proteins such as Cry1A, Cry3A) of US Patent Numbers 8,304,604, 8,304,605 and 8,476,226; Cry1B of US Patent Application Serial Number 10/525,318; Cry1C of US Patent Number 6,033,874; Cry1F of US Patent Numbers 5,188,960 and 6,218,188; Cry1A/F chimeras of US Patent Numbers 7,070,982; 6,962,705 and 6,713,063; a Cry2 protein such as Cry2Ab protein of US Patent Number 7,064,249; a Cry3A protein including but not limited to an engineered hybrid insecticidal protein (eHIP) created by fusing unique combinations of variable regions and conserved blocks of at least two different Cry proteins (US Patent Application Publication Number 2010/0017914); a Cry4 protein; a Cry5 protein; a Cry6 protein; Cry8 proteins of US Patent Numbers 7,329,736, 7,449,552, 7,803,943, 7,476,781, 7,105,332, 7,378,499 and 7,462,760; a Cry9 protein such as such as members of the Cry9A, Cry9B, Cry9C, Cry9D, Cry9E and Cry9F families; a Cry15 protein of Naimov, et al., (2008) Applied and Environmental Microbiology, 74:7145-7151; a Cry22, a Cry34Ab1 protein of US Patent Numbers 6,127,180, 6,624,145 and 6,340,593; a CryET33 and cryET34 protein of US Patent Numbers 6,248,535, 6,326,351, 6,399,330, 6,949,626, 7,385,107 and 7,504,229; a CryET33 and CryET34 homologs of US Patent Publication Number 2006/0191034, 2012/0278954, and PCT Publication Number WO 2012/139004; a Cry35Ab1 protein of US Patent Numbers 6,083,499, 6,548,291 and 6,340,593; a Cry46 protein, a Cry 51 protein, a Cry binary toxin; a TIC901 or related toxin; TIC807 of US Patent Application Publication Number 2008/0295207; ET29, ET37, TIC809, TIC810, TIC812, TIC127, TIC128 of PCT US 2006/033867; AXMI-027, AXMI-036, and AXMI-038 of US Patent Number 8,236,757; AXMI-031, AXMI-039, AXMI-040, AXMI-049 of US Patent Number 7,923,602; AXMI-018, AXMI-020 and AXMI-021 of WO 2006/083891; AXMI-010 of WO 2005/038032; AXMI-003 of WO 2005/021585; AXMI-008 of US Patent Application Publication Number 2004/0250311; AXMI-006 of US Patent Application Publication Number 2004/0216186; AXMI-007 of US Patent Application Publication Number 2004/0210965; AXMI-009 of US Patent Application Number 2004/0210964; AXMI-014 of US Patent Application Publication Number 2004/0197917; AXMI-004 of US Patent Application Publication Number 2004/0197916; AXMI-028 and AXMI-029 of WO 2006/119457; AXMI-007, AXMI-008, AXMI-008orf2, AXMI-009, AXMI-014 and AXMI-004 of WO 2004/074462; AXMI-150 of US Patent Number 8,084,416; AXMI-205 of US Patent Application Publication Number 2011/0023184; AXMI-011, AXMI-012, AXMI-013, AXMI-015, AXMI-019, AXMI-044, AXMI-037, AXMI-043, AXMI-033, AXMI-034, AXMI-022, AXMI-023, AXMI-041, AXMI-063 and AXMI-064 of US Patent Application Publication Number 2011/0263488; AXMI-R1 and related proteins of US Patent Application Publication Number 2010/0197592; AXMI221Z, AXMI222z, AXMI223z, AXMI224z and AXMI225z of WO 2011/103248; AXMI218, AXMI219, AXMI220, AXMI226, AXMI227, AXMI228, AXMI229, AXMI230 and AXMI231 of WO 2011/103247; AXMI-115, AXMI-113, AXMI-005, AXMI-163 and AXMI-184 of US Patent Number 8,334,431; AXMI-001, AXMI-002, AXMI-030,

AXMI-035 and AXMI-045 of US Patent Application Publication Number 2010/0298211; AXMI-066 and AXMI-076 of US Patent Application Publication Number 2009/0144852; AXMI128, AXMI130, AXMI131, AXMI133, AXMI140, AXMI141, AXMI142, AXMI143, AXMI144, AXMI146, AXMI148, AXMI149, AXMI152, AXMI153, AXMI154, AXMI155, AXMI156, AXMI157, AXMI158, AXMI162, AXMI165, AXMI166, AXMI167, AXMI168, AXMI169, AXMI170, AXMI171, AXMI172, AXMI173, AXMI174, AXMI175, AXMI176, AXMI177, AXMI178, AXMI179, AXMI180, AXMI181, AXMI182, AXMI185, AXMI186, AXMI187, AXMI188, AXMI189 of US Patent Number 8,318,900; AXMI079, AXMI080, AXMI081, AXMI082, AXMI091, AXMI092, AXMI096, AXMI097, AXMI098, AXMI099, AXMI100, AXMI101, AXMI102, AXMI103, AXMI104, AXMI107, AXMI108, AXMI109, AXMI110, AXMI111, AXMI112, AXMI114, AXMI116, AXMI117, AXMI118, AXMI119, AXMI120, AXMI121, AXMI122, AXMI123, AXMI124, AXMI1257, AXMI1268, AXMI127, AXMI129, AXMI164, AXMI151, AXMI161, AXMI183, AXMI132, AXMI138, AXMI137 of US Patent Application Publication Number 2010/0005543, cry proteins such as Cry1A and Cry3A having modified proteolytic sites of US Patent Number 8,319,019; a Cry1Ac, Cry2Aa and Cry1Ca toxin protein from *Bacillus thuringiensis* strain VBTS 2528 of US Patent Application Publication Number 2011/0064710. Other Cry proteins are well known to one skilled in the art (see, Crickmore, et al., "Bacillus thuringiensis toxin nomenclature" (2011), at lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ which can be accessed on the world-wide web using the "www" prefix). The insecticidal activity of Cry proteins is well known to one skilled in the art (for review, see, van Franckenhuyzen, (2009) J. Invert. Path. 101:1-16). The use of Cry proteins as transgenic plant traits is well known to one skilled in the art and Cry-transgenic plants including but not limited to plants expressing Cry1Ac, Cry1Ac+Cry2Ab, Cry1Ab, Cry1A.105, Cry1F, Cry1Fa2, Cry1F+Cry1Ac, Cry2Ab, Cry3A, mCry3A, Cry3Bb1, Cry34Ab1, Cry35Ab1, Vip3A, mCry3A, Cry9c and CBI-Bt have received regulatory approval (see, Sanahuja, (2011) Plant Biotech Journal 9:283-300 and the CERA. (2010) GM Crop Database Center for Environmental Risk Assessment (CERA), ILSI Research Foundation, Washington D.C. at cera-gmc.org/index.php?action=gm_crop_database which can be accessed on the world-wide web using the "www" prefix). More than one pesticidal proteins well known to one skilled in the art can also be expressed in plants such as Vip3Ab & Cry1Fa (US2012/0317682); Cry1BE & Cry1F (US2012/0311746); Cry1CA & Cry1AB (US2012/0311745); Cry1F & CryCa (US2012/0317681); Cry1DA & Cry1BE (US2012/0331590); Cry1DA & Cry1Fa (US2012/0331589); Cry1AB & Cry1BE (US2012/0324606); Cry1Fa & Cry2Aa and Cry1I & Cry1E (US2012/0324605); Cry34Ab/35Ab and Cry6Aa (US20130167269); Cry34Ab/Cry35Ab & Cry3Aa (US20130167268); and Cry3A and Cry1Ab or Vip3Aa (US20130116170). Pesticidal proteins also include insecticidal lipases including lipid acyl hydrolases of US Patent Number 7,491,869, and cholesterol oxidases such as from *Streptomyces* (Purcell et al. (1993) Biochem Biophys Res Commun 15:1406-1413). Pesticidal proteins also include VIP (vegetative insecticidal proteins) toxins of US Patent Numbers 5,877,012, 6,107,279 6,137,033, 7,244,820, 7,615,686, and 8,237,020. Other VIP proteins are well known to one skilled in the art (see, lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/vip.html which can be accessed on the worldwide web using the "www" prefix). Pesticidal proteins also include toxin complex (TC) proteins, obtainable from organisms such as *Xenorhabdus*, *Photorhabdus* and *Paenibacillus* (see, US Patent Numbers 7,491,698 and 8,084,418). Some TC proteins have "stand alone" insecticidal activity and other TC proteins enhance the activity of the stand-alone toxins produced by the same given organism. The toxicity of a "stand-alone" TC protein (from *Photorhabdus*, *Xenorhabdus* or *Paenibacillus*, for example) can be enhanced by one or more TC protein "potentiators" derived from a source organism of a different genus. There are three main types of TC proteins. As referred to herein, Class A proteins ("Protein A") are stand-alone toxins. Class B proteins ("Protein B") and Class C proteins ("Protein C") enhance the toxicity of Class A proteins. Examples of Class A proteins are TcbA, TcdA, XptA1 and XptA2. Examples of Class B proteins are TcaC, TcdB, XptB1Xb and XptC1Wi. Examples of Class C proteins are TccC, XptC1Xb and XptB1Wi. Pesticidal proteins also include spider, snake and scorpion venom proteins. Examples of spider venom peptides include but are not limited to lycotoxin-1 peptides and mutants thereof (US Patent Number 8,334,366).

[0028] In some embodiments the PtlP-50 polypeptide or the PtlP-65 polypeptide include amino acid sequences deduced from the full-length nucleic acid sequences disclosed herein and amino acid sequences that are shorter than the full-length sequences, either due to the use of an alternate downstream start site or due to processing that produces a shorter protein having pesticidal activity. Processing may occur in the organism the protein is expressed in or in the pest after ingestion of the protein.

[0029] Thus, provided herein are novel isolated or recombinant nucleic acid sequences that confer pesticidal activity. Also provided are the amino acid sequences of PtlP-50 polypeptides and PtlP-65 polypeptides. The protein resulting from translation of these PtlP-50 polypeptide and the PtlP-65 polypeptide genes allows cells to control or kill pests that ingest it.

Nucleic Acid Molecules, and Variants and Fragments Thereof

[0030] One aspect pertains to isolated or recombinant nucleic acid molecules comprising nucleic acid sequences encoding PtlP-50 polypeptides, PtlP-65 polypeptides or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify nucleic acid molecules encoding proteins with regions of sequence homology. As used herein, the term "nucleic acid molecule" refers to DNA molecules (e.g., recombinant DNA,

cDNA, genomic DNA, plastid DNA, mitochondrial DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

[0031] An "isolated" nucleic acid molecule (or DNA) is used herein to refer to a nucleic acid sequence (or DNA) that is no longer in its natural environment, for example in vitro. A "recombinant" nucleic acid molecule (or DNA) is used herein to refer to a nucleic acid sequence (or DNA) that is in a recombinant bacterial or plant host cell. In some embodiments, an "isolated" or "recombinant" nucleic acid is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For purposes of the disclosure, "isolated" or "recombinant" when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the recombinant nucleic acid molecule encoding PtIP-50 polypeptides or PtIP-65 polypeptides can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleic acid sequences that naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived.

[0032] In some embodiments an isolated nucleic acid molecule encoding PtIP-50 polypeptides or PtIP-65 polypeptides has one or more change in the nucleic acid sequence compared to the native or genomic nucleic acid sequence. In some embodiments the change in the native or genomic nucleic acid sequence includes but is not limited to: changes in the nucleic acid sequence due to the degeneracy of the genetic code; changes in the nucleic acid sequence due to the amino acid substitution, insertion, deletion and/or addition compared to the native or genomic sequence; removal of one or more intron; deletion of one or more upstream or downstream regulatory regions; and deletion of the 5' and/or 3' untranslated region associated with the genomic nucleic acid sequence. In some embodiments the nucleic acid molecule encoding a PtIP-50 polypeptide or PtIP-65 polypeptide is a non-genomic sequence.

[0033] A variety of polynucleotides that encode PtIP-50 polypeptides, PtIP-65 polypeptides or related proteins are contemplated. Such polynucleotides are useful for production of PtIP-50 polypeptides and PtIP-65 polypeptides in host cells when operably linked to suitable promoter, transcription termination and/or polyadenylation sequences. Such polynucleotides are also useful as probes for isolating homologous or substantially homologous polynucleotides that encode PtIP-50 polypeptides, PtIP-65 polypeptides or related proteins.

Polynucleotides encoding PtIP-50 polypeptides

[0034] One source of polynucleotides that encode PtIP-50 polypeptides or related proteins is a fern or other primitive plant species which contains a PtIP-50 polynucleotide encoding a PtIP-50 polypeptide of. The polynucleotides can be used to express PtIP-50 polypeptides in bacterial hosts that include but are not limited to *Agrobacterium*, *Bacillus*, *Escherichia*, *Salmonella*, *Pseudomonas* and *Rhizobium* bacterial host cells. The polynucleotides are also useful as probes for isolating homologous or substantially homologous polynucleotides that encode PtIP-50 polypeptides or related proteins. Such probes can be used to identify homologous or substantially homologous polynucleotides derived from *Pteridophyta* species.

[0035] Polynucleotides that encode PtIP-50 polypeptides can also be synthesized de novo from a PtIP-50 polypeptide sequence. The sequence of the polynucleotide gene can be deduced from a PtIP-50 polypeptide sequence through use of the genetic code. Computer programs such as "BackTranslate" (GCG™ Package, Accelrys, Inc. San Diego, Calif.) can be used to convert a peptide sequence to the corresponding nucleotide sequence encoding the peptide. Furthermore, synthetic PtIP-50 polynucleotide sequences of the disclosure can be designed so that they will be expressed in plants. US Patent Number 5,500,365 describes a method for synthesizing plant genes to improve the expression level of the protein encoded by the synthesized gene. This method relates to the modification of the structural gene sequences of the exogenous transgene, to cause them to be more efficiently transcribed, processed, translated and expressed by the plant. Features of genes that are expressed well in plants include elimination of sequences that can cause undesired intron splicing or polyadenylation in the coding region of a gene transcript while retaining substantially the amino acid sequence of the toxic portion of the insecticidal protein. A similar method for obtaining enhanced expression of transgenes in monocotyledonous plants is disclosed in US Patent Number 5,689,052.

[0036] "Complement" is used herein to refer to a nucleic acid sequence that is sufficiently complementary to a given nucleic acid sequence such that it can hybridize to the given nucleic acid sequence to thereby form a stable duplex. "Polynucleotide sequence variants" is used herein to refer to a nucleic acid sequence that except for the degeneracy of the genetic code encodes the same polypeptide.

[0037] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide or PtIP-65 polypeptide is a non-genomic nucleic acid sequence. As used herein a "non-genomic nucleic acid sequence" or "non-genomic nucleic acid molecule" or "non-genomic polynucleotide" refers to a nucleic acid molecule that has one or more change in the nucleic acid sequence compared to a native or genomic nucleic acid sequence. In some aspects the change to a native or genomic nucleic acid molecule includes but is not limited to: changes in the nucleic acid sequence due to the degeneracy of the genetic code; codon optimization of the nucleic acid sequence for expression in plants; changes in the nucleic

acid sequence to introduce at least one amino acid substitution, insertion, deletion and/or addition compared to the native or genomic sequence; removal of one or more intron associated with the genomic nucleic acid sequence; insertion of one or more heterologous introns; deletion of one or more upstream or downstream regulatory regions associated with the genomic nucleic acid sequence; insertion of one or more heterologous upstream or downstream regulatory regions; deletion of the 5' and/or 3' untranslated region associated with the genomic nucleic acid sequence; insertion of a heterologous 5' and/or 3' untranslated region; and modification of a polyadenylation site. In some embodiments the non-genomic nucleic acid molecule is a cDNA. In some aspects the non-genomic nucleic acid molecule is a synthetic nucleic acid sequence.

[0038] In some aspects the nucleic acid molecule encodes a PtIP-50 polypeptide comprising an amino acid sequence of having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70 or more amino acid substitutions compared to the native amino acid at the corresponding position.

[0039] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Division *Pteridophyta*. The phylogeny of ferns as used herein is based on the classification for extant ferns by A. R. Smith et al, TAXON, 55:705-731 (2006). The consensus phylogeny based on the classification by A. R. Smith is shown in Figure 1. Other phylogenetic classifications of extant ferns are known to one skilled in the art. Additional information on the phylogeny of ferns can be found at mobot.org/MOBOT/research/APweb/ (which can be accessed using the "www" prefix) and Schuettpelz E. and Pryer K. M., TAXON 56: 1037-1050 (2007) based on three plastid genes. Additional fern and other primitive plant species can be found at homepages.caverock.net.nz/~bj/fern/list.htm (which can be accessed using the http:// prefix).

[0040] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Psilotales*. In some aspects the the nucleic acid molecule encoding PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*, Family *Psilotaceae*. In some embodiments the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales* Family *Ophioglossaceae*. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Genus *Ophioglossum* L., *Botrychium*, *Botrypus*, *Helminthostachys*, *Ophioderma*, *Cheiroglossa*, *Sceptridium* or *Mankyua*. In some embodiments the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the *Ophioglossum* L. Genus is selected from but not limited to *Ophioglossum californicum*, *Ophioglossum coriaceum*, *Ophioglossum costatum*, *Ophioglossum crotalophoroides*, *Ophioglossum engelmannii*, *Ophioglossum falcatum*, *Ophioglossum gomezianum*, *Ophioglossum gramineum*, *Ophioglossum kawamurae*, *Ophioglossum lusitanicum*, *Ophioglossum namegatae*, *Ophioglossum nudicaule*, *Ophioglossum palmatum*, *Ophioglossum parvum*, *Ophioglossum pedunculatum*, *Ophioglossum pendulum*, *Ophioglossum petiolatum*, *Ophioglossum pusillum*, *Ophioglossum reticulatum*, *Ophioglossum richardsiae*, *Ophioglossum thermale*, and *Ophioglossum vulgatum*.

[0041] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a species in the Class *Polypodiopsida/Pteridopsida*. In some embodiments the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order *Osmundales* (royal ferns); Family *Osmundaceae*. In some embodiments the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order *Hymenophyllales* (filmy ferns and bristle ferns); Family *Hymenophyllaceae*. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order *Gleicheniales*; Family *Gleicheniaceae*, Family *Dipteridaceae* or Family *Matoniaceae*. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order Schizaeales; Family *Lygodiaceae*, Family *Anemiaceae* or Family *Schizaeaceae*. In some embodiments the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order Salviniales; Family *Marsileaceae* or Family *Salviniaceae*. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order *Cyatheaales*; Family *Thyrsopteridaceae*, Family *Loxsomataceae*, Family *Culcitaceae*, Family *Plagiogyriaceae*, Family *Cibotiaceae*, Family *Cyatheaceae*, Family *Dicksoniaceae* or Family *Metaxyaceae*.

[0042] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales; Family *Lindsaeaceae*, Family *Saccolomataceae*, Family *Cystodiaceae*, Family *Dennstaedtiaceae*, Family *Pteridaceae*, Family *Aspleniaceae*, Family *Thelypteridaceae*, Family *Woodsiaceae*, Family *Onocleaceae*, Family *Blechnaceae*, Family *Dryopteridaceae*, Family *Lomariopsidaceae*, Family *Tectariaceae*, Family *Oleandraceae*, Family *Davalliaceae* or Family *Polypodiaceae*.

[0043] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Pteridaceae*, Genus *Adiantaceae* selected from but not limited to *Adiantum aethiopicum*, *Adiantum aleuticum*, *Adiantum bonatianum*, *Adiantum cajennense*, *Adiantum capillus-junonis*, *Adiantum capillus-veneris*, *Adiantum caudatum*, *Adiantum chienii*, *Adiantum chilense*, *Adiantum cuneatum*, *Adiantum cunninghamii*, *Adi-*

antum davidii, *Adiantum diaphanum*, *Adiantum edentulum*, *Adiantum edgeworthii*, *Adiantum excisum*, *Adiantum fengianum*, *Adiantum fimbriatum*, *Adiantum flabellulatum*, *Adiantum formosanum*, *Adiantum formosum*, *Adiantum fulvum*, *Adiantum gravesii*, *Adiantum hispidulum*, *Adiantum induratum*, *Adiantum jordanii*, *Adiantum juxtapositum*, *Adiantum latifolium*, *Adiantum leveillei*, *Adiantum lianxianense*, *Adiantum malesianum*, *Adiantum mariesii*, *Adiantum monochlamys*, *Adiantum myriosorum*, *Adiantum obliquum*, *Adiantum ogasawarensense*, *Adiantum pedatum*, *Adiantum pentadactylon*, *Adiantum peruvianum*, *Adiantum philippense*, *Adiantum princeps*, *Adiantum pubescens*, *Adiantum raddianum*, *Adiantum reniforme*, *Adiantum roborowskii*, *Adiantum serratodentatum*, *Adiantum sinicum*, *Adiantum soboliferum*, *Adiantum subcordatum*, *Adiantum tenerum*, *Adiantum terminatum*, *Adiantum tetraphyllum*, *Adiantum venustum*, *Adiantum viridescens*, and *Adiantum viridimontanum*.

[0044] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Aspleniaceae, Genus *Asplenium* L. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Aspleniaceae, Genus *Asplenium* L selected from but not limited to *Asplenium abbreviatum*, *Asplenium abrotanoides*, *Asplenium abscissum* var. *subaequilaterale*, *Asplenium abscissum*, *Asplenium achilleifolium*, *Asplenium acuminatum*, *Asplenium adiantifrons*, *Asplenium adiantoides*, *Asplenium adiantoides* var. *squamulosum*, *Asplenium adiantum-nigrum* L., *Asplenium adiantum-nigrum* var. *adiantum-nigrum*, *Asplenium adiantum-nigrum* var. *yuanum*, *Asplenium adnatum*, *Asplenium aethiopicum*, *Asplenium affine*, *Asplenium affine* var. *affine*, *Asplenium affine* var. *gilpinae*, *Asplenium affine* var. *mettenii*, *Asplenium affine* var. *pecten*, *Asplenium africanum*, *Asplenium afzelii*, *Asplenium aitchisonii*, *Asplenium alatum*, *Asplenium alatum*, *Asplenium alfredii*, *Asplenium altajense*, *Asplenium amabile*, *Asplenium ambohitantelense*, *Asplenium anceps* var. *proliferum*, *Asplenium andapense*, *Asplenium andersonii*, *Asplenium angustatum*, *Asplenium angustum*, *Asplenium anisophyllum*, *Asplenium annetii*, *Asplenium antiquum*, *Asplenium antrophyoides*, *Asplenium apertum*, *Asplenium apogonum*, *Asplenium aquaticum*, *Asplenium arboreum*, *Asplenium arcanum*, *Asplenium arcuatum*, *Asplenium argentinum*, *Asplenium argutum*, *Asplenium aspidiiforme*, *Asplenium aspidioides*, *Asplenium asterolepis*, *Asplenium auricularium* var. *acutidens*, *Asplenium auricularium* var. *subintegerrimum*, *Asplenium auriculatum*, *Asplenium auriculatum* var. *aequilaterale*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *nana*, *Asplenium auritum*, *Asplenium auritum* var. *auriculatum*, *Asplenium auritum* var. *auritum*, *Asplenium auritum* var. *bipinnatifidum*, *Asplenium auritum* var. *bipinnatisectum*, *Asplenium auritum* var. *davallioides*, *Asplenium auritum* var. *macilentum*, *Asplenium auritum* var. *rigidum*, *Asplenium auritum* var. *subsimplex*, *Asplenium austrochinense*, *Asplenium ayopayense*, *Asplenium badinii*, *Asplenium balense*, *Asplenium ballivianii*, *Asplenium bangii*, *Asplenium bangii*, *Asplenium barbaense*, *Asplenium barclayanum*, *Asplenium barkamense*, *Asplenium barteri*, *Asplenium basispicum*, *Asplenium bicrenatum*, *Asplenium bifrons*, *Asplenium bipartitum*, *Asplenium blastophorum*, *Asplenium blepharodes*, *Asplenium blepharophorum*, *Asplenium boiteaui*, *Asplenium bolivianum*, *Asplenium boltonii*, *Asplenium borealichinense*, *Asplenium bradei*, *Asplenium bradeorum*, *Asplenium bradleyi*, *Asplenium brausei*, *Asplenium breedlovei*, *Asplenium buettneri*, *Asplenium buettneri* var. *hildebrandtii*, *Asplenium bulbiferum*, *Asplenium bullatum* var. *bullatum*, *Asplenium bullatum* var. *shikokianum*, *Asplenium bullatum*, *Asplenium cancellatum*, *Asplenium capillipes*, *Asplenium cardiophyllum* (Hance), *Asplenium caripense*, *Asplenium carvalhoanum*, *Asplenium castaneoviride*, *Asplenium castaneum*, *Asplenium caudatum*, *Asplenium celtidifolium* (Kunze), *Asplenium ceratolepis*, *Asplenium changputungense*, *Asplenium chaseanum*, *Asplenium cheilosorum*, *Asplenium chengkouense*, *Asplenium chihuahuaense*, *Asplenium chimantae*, *Asplenium chimborazense*, *Asplenium chingianum*, *Asplenium chlorophyllum*, *Asplenium chondrophyllum*, *Asplenium cicutarium*, *Asplenium cicutarium* var. *paleaceum*, *Asplenium cirrhatum*, *Asplenium cladolepton*, *Asplenium clausenii*, *Asplenium coenobiale*, *Asplenium commutatum*, *Asplenium congestum*, *Asplenium conquisitum*, *Asplenium consimile*, *Asplenium contiguum*, *Asplenium contiguum* var. *hirtulum*, *Asplenium corderoi*, *Asplenium cordovense*, *Asplenium coriaceum*, *Asplenium coriifolium*, *Asplenium correardii*, *Asplenium costale*, *Asplenium costale* var. *robustum*, *Asplenium cowanii*, *Asplenium crenulatoserrulatum*, *Asplenium crenulatum*, *Asplenium crinicaule*, *Asplenium crinulosum*, *Asplenium cristatum*, *Asplenium cryptolepis* Fernald, *Asplenium cultrifolium* L., *Asplenium cuneatiforme*, *Asplenium cuneatum*, *Asplenium curvatum*, *Asplenium cuspidatum*, *Asplenium cuspidatum* var. *cuspidatum*, *Asplenium cuspidatum* var. *foeniculaceum*, *Asplenium cuspidatum* var. *triculum*, *Asplenium cuspidatum* var. *tripinnatum*, *Asplenium dalhousiae*, *Asplenium dareoides*, *Asplenium davallioides*, *Asplenium davisii*, *Asplenium debile*, *Asplenium debile*, *Asplenium decussatum*, *Asplenium delavayi*, *Asplenium delicatulum*, *Asplenium delicatulum* var. *cocosensis*, *Asplenium delitescens*, *Asplenium delitescens* X *laetum*, *Asplenium densum*, *Asplenium dentatum* L., *Asplenium dentatum* L., *Asplenium depauperatum*, *Asplenium deqenense*, *Asplenium diana*, *Asplenium difforme*, *Asplenium dilatatum*, *Asplenium dimidiatum*, *Asplenium dimidiatum* var. *bolivense*, *Asplenium diplazisorum*, *Asplenium dissectum*, *Asplenium distans*, *Asplenium divaricatum*, *Asplenium divergens*, *Asplenium divisissimum*, *Asplenium doederleinii*, *Asplenium donnell-smithii*, *Asplenium dregeanum*, *Asplenium du-longjiangense*, *Asplenium duplicatoserratum*, *Asplenium eatonii*, *Asplenium ebeneum*, *Asplenium ebenoides*, *Asplenium ecuadorensense*, *Asplenium eggersii*, *Asplenium emarginatum*, *Asplenium enatum*, *Asplenium ensiforme* fo. *bicuspe*, *Asplenium ensiforme* fo. *ensiforme*, *Asplenium ensiforme* fo. *stenophyllum*, *Asplenium ensiforme*, *Asplenium erectum* var. *erectum*, *Asplenium erectum* var. *gracile*, *Asplenium erectum* var. *usambarensense*, *Asplenium erectum* var. *zeyheri*, & *Asplenium erosum* L., *Asplenium escaleroense*, *Asplenium esculentum*, *Asplenium eutecnum*, *Asplenium excelsum*,

Asplenium excisum, *Asplenium exiguum*, *Asplenium extensum*, *Asplenium falcatum*, *Asplenium falcinellum*, *Asplenium faurei*, *Asplenium feei*, *Asplenium fengyangshanense*, *Asplenium ferulaceum*, *Asplenium fibrillosum*, *Asplenium filix-femina*, *Asplenium finckii*, *Asplenium finlaysonianum*, *Asplenium flabellulatum*, *Asplenium flabellulatum* var. *flabellulatum*, *Asplenium flabellulatum* var. *partitum*, *Asplenium flaccidum*, *Asplenium flavescens*, *Asplenium flavidum*, *Asplenium flexuosum*, *Asplenium fluminense*, *Asplenium foeniculaceum*, *Asplenium formosanum*, *Asplenium formosum* var. *carolinum*, *Asplenium formosum* var. *incultum*, *Asplenium formosum*, *Asplenium fournieri*, *Asplenium fragile*, *Asplenium fragile* var. *lomense*, *Asplenium fragrans*, *Asplenium fragrans* var. *foeniculaceum*, *Asplenium franconis* var. *gracile*, *Asplenium fraxinifolium*, *Asplenium friesiorum*, *Asplenium friesiorum* var. *nesophilum*, *Asplenium fugax*, *Asplenium fujianense*, *Asplenium furcatum*, *Asplenium furfuraceum*, *Asplenium fuscipes*, *Asplenium fuscopubescens*, *Asplenium galeottii*, *Asplenium gautieri*, *Asplenium gemmiferum*, *Asplenium gentryi*, *Asplenium geppii*, *Asplenium ghiesbreghtii*, *Asplenium gilliesii*, *Asplenium gilpinae*, *Asplenium glanduliserratum*, *Asplenium glenniei*, *Asplenium goldmannii*, *Asplenium gomezianum*, *Asplenium grande*, *Asplenium grandifolium*, *Asplenium grandifrons*, *Asplenium gregoriae*, *Asplenium griffithianum*, *Asplenium gulingense*, *Asplenium hainanense*, *Asplenium hallbergii*, *Asplenium hallei*, *Asplenium hallii*, *Asplenium hangzhouense*, *Asplenium haplophyllum*, *Asplenium harpeodes*, *Asplenium harpeodes* var. *glaucovirens*, *Asplenium harpeodes* var. *incisum*, *Asplenium harrisii* Jenman, *Asplenium harrisonii*, *Asplenium hastatum*, *Asplenium hebeiense*, *Asplenium hemionitideum*, *Asplenium hemitomum*, *Asplenium henryi*, *Asplenium herpetopteris*, *Asplenium herpetopteris* var. *herpetopteris*, *Asplenium herpetopteris* var. *acutipinnata*, *Asplenium herpetopteris* var. *masoulae*, *Asplenium herpetopteris* var. *villosum*, *Asplenium hesperium*, *Asplenium heterochroum*, *Asplenium hians*, *Asplenium hians* var. *pallescens*, *Asplenium hoffmannii*, *Asplenium holophlebium*, *Asplenium hondoense*, *Asplenium horridum*, *Asplenium hostmannii*, *Asplenium humistratum*, *Asplenium hypomelas*, *Asplenium inaequilaterale*, *Asplenium incisum*, *Asplenium incurvatum*, *Asplenium indicum*, *Asplenium indicum* var. *indicum*, *Asplenium indicum* var. *yoshingagae*, *Asplenium induratum*, *Asplenium indusiatum*, *Asplenium inexpectatum*, *Asplenium insigne*, *Asplenium insiticium*, *Asplenium insolitum*, *Asplenium integerrimum*, *Asplenium interjectum*, *Asplenium jamesonii*, *Asplenium jaundeense*, *Asplenium juglandifolium*, *Asplenium kangdingense*, *Asplenium kansuense*, *Asplenium kassneri*, *Asplenium kaulfussii*, *Asplenium kellermanii*, *Asplenium kentuckiense*, *Asplenium khullarii*, *Asplenium kiangsuense*, *Asplenium kunzeanum*, *Asplenium lacerum*, *Asplenium laciniatum*, *Asplenium laciniatum* var. *acutipinna*, *Asplenium laciniatum* var. *laciniatum*, *Asplenium laetum* fo. *minor*, *Asplenium laetum*, *Asplenium laetum* var. *incisoserratum*, *Asplenium lamprocaulon*, *Asplenium laserpitifolium* var. *morrisonense*, *Asplenium lastii*, *Asplenium latedens*, *Asplenium latifolium*, *Asplenium laui*, *Asplenium laurentii*, *Asplenium leandrianum*, *Asplenium lechleri*, *Asplenium leiboense*, *Asplenium lepidorachis*, *Asplenium leptochlamys*, *Asplenium leptophyllum*, *Asplenium levyi*, *Asplenium lindbergii*, *Asplenium lindeni*, *Asplenium lineatum*, *Asplenium lividum*, *Asplenium lobatum*, *Asplenium lobulatum*, *Asplenium lokohoense*, *Asplenium longicauda*, *Asplenium longicaudatum*, *Asplenium longifolium*, *Asplenium longisorum*, *Asplenium longjinense*, *Asplenium lorentzii*, *Asplenium loriceum*, *Asplenium loxogrammoides*, *Asplenium lugubre*, *Asplenium lunulatum*, *Asplenium lunulatum* var. *pteropus*, *Asplenium lushanense*, *Asplenium lydgatei*, *Asplenium macilentum*, *Asplenium macraei*, *Asplenium macrodictyon*, *Asplenium macrophlebium*, *Asplenium macrophyllum*, *Asplenium macropterum*, *Asplenium macrosorum*, *Asplenium macrotis*, *Asplenium macrurum*, *Asplenium mainlingense*, *Asplenium mangindranense*, *Asplenium mannii*, *Asplenium marginatum* L., *Asplenium marojejense*, *Asplenium martianum*, *Asplenium matsumurae*, *Asplenium mauritiensis* Lorence, *Asplenium maximum*, *Asplenium*, ii, *Asplenium megalura*, *Asplenium megaphyllum*, *Asplenium meiotomum*, *Asplenium melanopus*, *Asplenium membranifolium*, *Asplenium meniscioides*, *Asplenium mesosorum*, *Asplenium mexicanum*, *Asplenium micropaleatum*, *Asplenium microtum*, *Asplenium mildbraedii*, *Asplenium mildei*, *Asplenium minimum*, *Asplenium minutum*, *Asplenium miradoreense*, *Asplenium miyunense*, *Asplenium mocceanum*, *Asplenium mocquersii*, *Asplenium modestum*, *Asplenium monanthes* var. *menziesii*, *Asplenium monanthes* L., *Asplenium monanthes* var. *monanthes*, *Asplenium monanthes* var. *castaneum*, *Asplenium monanthes* var. *wagneri*, *Asplenium monanthes* var. *yungense*, *Asplenium monodon*, *Asplenium montanum*, *Asplenium mosetenense*, *Asplenium moupinense*, *Asplenium mucronatum*, *Asplenium munchii*, *Asplenium muticum*, *Asplenium myapteron*, *Asplenium myriophyllum*, *Asplenium nakanoanum*, *Asplenium nanchuanense*, *Asplenium nemorale*, *Asplenium neolaserpitifolium*, *Asplenium neomutijugum*, *Asplenium neovarians*, *Asplenium nesii*, *Asplenium nesioticum*, *Asplenium nidus* L., *Asplenium nigricans*, *Asplenium niponicum*, *Asplenium normale*, *Asplenium normale* var. *angustum*, *Asplenium obesum*, *Asplenium oblongatum*, *Asplenium oblongifolium*, *Asplenium obovatum*, *Asplenium obscurum*, *Asplenium obscurum* var. *angustum*, *Asplenium obtusatum* var. *obtusatum*, *Asplenium obtusatum* var. *sphenoides*, *Asplenium obtusifolium* L., *Asplenium obtusissimum*, *Asplenium obversum*, *Asplenium ochraceum*, *Asplenium oellgaardii*, *Asplenium ofeliae*, *Asplenium oldhami*, *Asplenium oligosorum*, *Asplenium olivaceum*, *Asplenium onopteris* L., *Asplenium onustum*, *Asplenium ortegae*, *Asplenium otites*, *Asplenium palaciosii*, *Asplenium palmeri*, *Asplenium partitum*, *Asplenium parvisorum*, *Asplenium parvisculum*, *Asplenium parvulum*, *Asplenium patens*, *Asplenium paucifolium*, *Asplenium paucijugum*, *Asplenium paucivenosum*, *Asplenium pearcei*, *Asplenium pekinense*, *Asplenium pellucidum*, *Asplenium pendulum*, *Asplenium petiolulatum*, *Asplenium phyllitidis*, *Asplenium pimpinellifolium*, *Asplenium pinnatifidum*, *Asplenium pinnatum*, *Asplenium platyneuron*, *Asplenium platyneuron* var. *bacculum-rubrum*, *Asplenium platyneuron* var. *incisum*, *Asplenium platyphyllum*, *Asplenium plumbeum*, *Asplenium poloense*, *Asplenium polymeris*, *Asplenium polymorphum*, *Asplenium polyodon*, *Asplenium poly-*

odon var. *knudsenii*, *Asplenium polyodon* var. *nitidulum*, *Asplenium polyodon* var. *sectum*, *Asplenium polyodon* var. *subcaudatum*, *Asplenium polyphyllum*, *Asplenium poolii*, *Asplenium poolii* fo. *simplex*, *Asplenium poolii* var. *linearipinnatum*, *Asplenium potosinum*, *Asplenium potosinum* var. *incisum*, *Asplenium praegracile*, *Asplenium praemorsum*, *Asplenium preussii*, *Asplenium pringleanum*, *Asplenium pringlei*, *Asplenium prionitis*, *Asplenium procerum*, *Asplenium progrediens*, *Asplenium projectum*, *Asplenium prolongatum*, *Asplenium propinquum*, *Asplenium protensum*, *Asplenium pseudoangustum*, *Asplenium pseudoerectum*, *Asplenium pseudofontanum*, *Asplenium pseudolaserpitiifolium*, *Asplenium pseudonormale*, *Asplenium pseudopellucidum*, *Asplenium pseudopraemorsum*, *Asplenium pseudovarians*, *Asplenium pseudowilfordii*, *Asplenium pseudowrightii*, *Asplenium psilacrum*, *Asplenium pteropus*, *Asplenium pubirhizoma*, *Asplenium pulchellum*, *Asplenium pulchellum* var. *subhorizontale*, *Asplenium pulcherrimum*, *Asplenium pulicosum*, *Asplenium pulicosum* var. *maius*, *Asplenium pululahuae*, *Asplenium pumilum*, *Asplenium pumilum* var. *hymenophylloides*, *Asplenium pumilum* var. *laciniatum*, *Asplenium purdieanum*, *Asplenium purpurascens*, *Asplenium pyramidatum*, *Asplenium qiujiangense*, *Asplenium quercicola*, *Asplenium quitense*, *Asplenium raddianum*, *Asplenium radiatum*, *Asplenium radicans* L., *Asplenium radicans*, *Asplenium radicans* var. *costaricense*, *Asplenium radicans* var. *partitum*, *Asplenium radicans* var. *radicans*, *Asplenium radicans* var. *uniseriale*, *Asplenium recumbens*, *Asplenium reflexum*, *Asplenium regulare* var. *latior*, *Asplenium repandulum*, *Asplenium repens*, *Asplenium repente*, *Asplenium resiliens*, *Asplenium retusulum*, *Asplenium rhipidoneuron*, *Asplenium rhizophorum* L., *Asplenium rhizophyllum*, *Asplenium rhizophyllum* L., *Asplenium rhizophyllum* var. *proliferum*, *Asplenium rhomboideum*, *Asplenium rigidum*, *Asplenium riparium*, *Asplenium rivale*, *Asplenium rockii*, *Asplenium roemerianum*, *Asplenium roemerianum* var. *mindensis*, *Asplenium rosenstockianum*, *Asplenium rubinum*, *Asplenium ruizianum*, *Asplenium rusbyanum*, *Asplenium ruta-muraria* L., *Asplenium ruta-muraria* var. *cryptolepis*, *Asplenium rutaceum*, *Asplenium rutaceum* var. *disculiferum*, *Asplenium rutaefolium*, *Asplenium rutifolium*, *Asplenium salicifolium* L., *Asplenium salicifolium* var. *aequilaterale*, *Asplenium salicifolium* var. *salicifolium*, *Asplenium sampsoni*, *Asplenium sanchezii*, *Asplenium sanderi*, *Asplenium sandersonii*, *Asplenium sanguinolentum*, *Asplenium sarelii*, *Asplenium sarelii* var. *magnum*, *Asplenium sarelii* var. *sarelii*, *Asplenium saxicola*, *Asplenium scalifolium*, *Asplenium scandicinum*, *Asplenium schizophyllum*, *Asplenium schkuhrii*, *Asplenium sciadophilum*, *Asplenium scolopendrium* L., *Asplenium scortechinii*, *Asplenium seileri*, *Asplenium semipinnatum*, *Asplenium septentrionale*, *Asplenium serra*, *Asplenium serra* var. *imrayanum*, *Asplenium serratissimum*, *Asplenium serratum* L., *Asplenium serratum* var. *caudatum*, *Asplenium serricula*, *Asplenium sessilifolium*, *Asplenium sessilifolium* var. *guatemalense*, *Asplenium sessilifolium* var. *minus*, *Asplenium sessilifolium* var. *occidentale*, *Asplenium sessilipinnum*, *Asplenium setosum*, *Asplenium shepherdii*, *Asplenium shepherdii* var. *bipinnatum*, *Asplenium shepherdii* var. *flagelliferum*, *Asplenium shikokianum*, *Asplenium simii*, *Asplenium simonsianum*, *Asplenium sintensisii*, *Asplenium skinneri*, *Asplenium skinneri*, *Asplenium sodiroi*, *Asplenium soleirolloides*, *Asplenium solidum* var. *stenophyllum*, *Asplenium solmsii*, *Asplenium* sp.-N.-Halle-2234, *Asplenium spathulinum*, *Asplenium spectabile*, *Asplenium speluncae*, *Asplenium sphaerosporum*, *Asplenium sphenotomum*, *Asplenium spinescens*, *Asplenium splendens*, *Asplenium sprucei*, *Asplenium squamosum* L., *Asplenium standleyi*, *Asplenium stellatum*, *Asplenium stenocarpum*, *Asplenium stoloniferum*, *Asplenium stolonipes*, *Asplenium striatum* L., *Asplenium stuebelianum*, *Asplenium stuhlmannii*, *Asplenium suave*, *Asplenium subalatum*, *Asplenium subcrenatum*, *Asplenium subdigitatum*, *Asplenium subdimidiatum*, *Asplenium subintegrum*, *Asplenium sublaserpitiifolium*, *Asplenium sublongum*, *Asplenium subnudum*, *Asplenium suborbiculare*, *Asplenium subtenuifolium*, *Asplenium subtile*, *Asplenium subtoramanum*, *Asplenium subtrapezoideum*, *Asplenium subvarians*, *Asplenium sulcatum*, *Asplenium sylvaticum*, *Asplenium szechuanense*, *Asplenium taiwanense*, *Asplenium tenerrimum*, *Asplenium tenerum*, *Asplenium tenuicaule*, *Asplenium tenuifolium*, *Asplenium tenuifolium* var. *minor*, *Asplenium tenuifolium* var. *tenuifolium*, *Asplenium tenuissimum*, *Asplenium ternatum*, *Asplenium theciferum*, *Asplenium theciferum* var. *concinnum*, *Asplenium thunbergii*, *Asplenium tianmushanense*, *Asplenium tianshanense*, *Asplenium tibeticum*, *Asplenium tocoraniense*, *Asplenium toramanum*, *Asplenium trapezoideum*, *Asplenium tricholepis*, *Asplenium trichomanes* L., *Asplenium trichomanes* subsp. *inexpectans*, *Asplenium trichomanes* subsp. *quadri-valens*, *Asplenium trichomanes* subsp. *trichomanes*, *Asplenium trichomanes* var. *harovii*, *Asplenium trichomanes* var. *herbaceum*, *Asplenium trichomanes* var. *repens*, *Asplenium trichomanes* var. *viridissimum*, *Asplenium trichomanes-dentatum* L., *Asplenium trigonopterum*, *Asplenium trilobatum*, *Asplenium trilobum*, *Asplenium triphyllum*, *Asplenium triphyllum* var. *compactum*, *Asplenium triphyllum* var. *gracillimum*, *Asplenium triphyllum* var. *herbaceum*, *Asplenium tripteropus*, *Asplenium triquetrum*, *Asplenium truncorum*, *Asplenium tsaratananense*, *Asplenium tucumanense*, *Asplenium tuerckheimii*, *Asplenium tunquiniense*, *Asplenium ulbrichtii*, *Asplenium ultimum*, *Asplenium unilaterale*, *Asplenium unilaterale* var. *decurrens*, *Asplenium unilaterale* var. *udum*, *Asplenium unilaterale* var. *unilaterale*, *Asplenium uniseriale*, *Asplenium uropterum*, *Asplenium vagans*, *Asplenium vareschianum*, *Asplenium variabile* var. *paucijugum*, *Asplenium variabile* var. *variabile*, *Asplenium varians* subsp. *fimbriatum*, *Asplenium varians*, *Asplenium vastum*, *Asplenium venturae*, *Asplenium venulosum*, *Asplenium verapax*, *Asplenium vesiculosum*, *Asplenium vespertinum*, *Asplenium villosum*, *Asplenium virens*, *Asplenium viride*, *Asplenium viridifrons*, *Asplenium virillae*, *Asplenium viviparioides*, *Asplenium viviparum*, *Asplenium viviparum* var. *viviparum*, *Asplenium viviparum* var. *lineatu*, *Asplenium volubile*, *Asplenium vulcanicum*, *Asplenium wacketii*, *Asplenium wagneri*, *Asplenium wallichianum*, *Asplenium warneckeii*, *Asplenium wilfordii*, *Asplenium williamsii*, *Asplenium wrightii*, *Asplenium wrightioides*, *Asplenium wuliangshanense*, *Asplenium xianqianense*, *Asplenium xinjiangense*, *Asplenium xinyiense*, *Asplenium*

yelagagense, *Asplenium yoshinagae*, *Asplenium yunnanense*, *Asplenium zamiifolium*, *Asplenium zanzibaricum*, *Asplenium biscayneanum*, *Asplenium curtissii*, *Asplenium ebenoides*, *Asplenium herb-wagneri*, *Asplenium heteroresiliens*, *Asplenium kenzoii*, *Asplenium plenum*, *Asplenium wangii*, and *Asplenium* × *clermontiae*, *Asplenium* × *gravesii*.

[0045] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Blechnaceae*, Genus *Blechnum* L. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order *Polypodiales*, Family *Blechnaceae*, Genus *Blechnum* L. selected from but not limited to *Blechnum amabile*, *Blechnum appendiculatum*, *Blechnum articulatum*, *Blechnum australe*, *Blechnum austrobrasilianum*, *Blechnum binervatum*, *Blechnum blechnoides*, *Blechnum brasiliense*, *Blechnum capense*, *Blechnum cartilagineum*, *Blechnum castaneum*, *Blechnum chambersii*, *Blechnum chilense*, *Blechnum colensoi*, *Blechnum contiguum*, *Blechnum cordatum*, *Blechnum coriaceum*, *Blechnum discolor*, *Blechnum doodioides*, *Blechnum durum*, *Blechnum eburneum*, *Blechnum ensiforme*, *Blechnum filiforme*, *Blechnum fluviatile*, *Blechnum fragile*, *Blechnum fraseri*, *Blechnum fullagari*, *Blechnum gibbum*, *Blechnum glandulosum*, *Blechnum gracile*, *Blechnum hancockii*, *Blechnum hastatum*, *Blechnum howeanum*, *Blechnum indicum*, *Blechnum kunthianum*, *Blechnum laevigatum*, *Blechnum loxense*, *Blechnum magellanicum*, *Blechnum membranaceum*, *Blechnum microbasis*, *Blechnum microphyllum*, *Blechnum milnei*, *Blechnum minus*, *Blechnum mochaenum*, *Blechnum montanum*, *Blechnum moorei*, *Blechnum moritzianum*, *Blechnum nigrum*, *Blechnum niponicum*, *Blechnum norfolkianum*, *Blechnum novae-zelandiae*, *Blechnum nudum*, *Blechnum obtusatum*, *Blechnum occidentale*, *Blechnum oceanicum*, *Blechnum orientale*, *Blechnum patersonii*, *Blechnum penna-marina*, *Blechnum polypodioides*, *Blechnum procerum*, *Blechnum punctulatum*, *Blechnum sampaioanum*, *Blechnum schiedeanum*, *Blechnum schomburgkii*, *Blechnum serrulatum*, *Blechnum simillimum*, *Blechnum spicant*, *Blechnum stipitellatum*, *Blechnum tabulare*, *Blechnum triangularifolium*, *Blechnum vieillardii*, *Blechnum vulcanicum*, *Blechnum wattsi*, *Blechnum whelanii*, and *Blechnum wurunuran*.

[0046] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Dryopteridaceae* Genus *Acrophorus*, Genus *Acrorumohra*, Genus *Anapausia*, Genus *Arachniodes*, Genus *Bolbitis*, Genus *Ctenitis*, Genus *Cyclodium*, Genus *Cyrtogonellum*, Genus *Cyrtomidictyum*, Genus *Cyrtomium*, Genus *Diacalpe*, Genus *Didymochlaena*, Genus *Dryopsis*, Genus *Dryopteris*, Genus *Elaphoglossum*, Genus *Hypodematum*, Genus *Lastreopsis*, Genus *Leptorumohra*, Genus *Leucostegia*, Genus *Lithostegia*, Genus *Lomagrumma*, Genus *Maxonia*, Genus *Megalastrum*, Genus *Olfersia*, Genus *Peranema*, Genus *Phanerophlebia*, Genus *Phanerophlebiopsis*, Genus *Polybotrya*, Genus *Polystichopsis*, Genus *Polystichum*, Genus *Rumohra*, Genus *Sorolepidium*, Genus *Stigmatopteris* or Genus *Teratophyllum*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Dryopteridaceae*, Genus *Bolbitis*, selected from but not limited to *Bolbitis acrostichoides*, *Bolbitis aliena*, *Bolbitis angustipinna*, *Bolbitis appendiculata*, *Bolbitis auriculata*, *Bolbitis bernoullii*, *Bolbitis bipinnatifida*, *Bolbitis cadieri*, *Bolbitis christensenii*, *Bolbitis confertifolia*, *Bolbitis costata*, *Bolbitis crispatula*, *Bolbitis fluviatilis*, *Bolbitis gaboensis*, *Bolbitis gemmifera*, *Bolbitis hainanensis*, *Bolbitis hastata*, *Bolbitis hekouensis*, *Bolbitis hemiotis*, *Bolbitis heteroclita*, *Bolbitis heudelotii*, *Bolbitis humblotii*, *Bolbitis interlineata*, *Bolbitis latipinna*, *Bolbitis laxireticulata*, *Bolbitis lindigii*, *Bolbitis lonchophora*, *Bolbitis longiflagellata*, *Bolbitis major*, *Bolbitis media*, *Bolbitis nicotianifolia*, *Bolbitis nodiflora*, *Bolbitis novoguineensis*, *Bolbitis oligarchica*, *Bolbitis palustris*, *Bolbitis pandurifolia*, *Bolbitis pergamentacea*, *Bolbitis portoricensis*, *Bolbitis presliana*, *Bolbitis quoyana*, *Bolbitis rawsonii*, *Bolbitis repanda*, *Bolbitis rhizophylla*, *Bolbitis riparia*, *Bolbitis rivularis*, *Bolbitis sagenioides*, *Bolbitis salicina*, *Bolbitis scalpturata*, *Bolbitis scandens*, *Bolbitis semicordata*, *Bolbitis semipinnatifida*, *Bolbitis serrata*, *Bolbitis serratifolia*, *Bolbitis simplex*, *Bolbitis sinensis*, *Bolbitis singaporensis*, *Bolbitis sinuata*, *Bolbitis subcordata*, *Bolbitis subcrenata*, *Bolbitis taylorii*, *Bolbitis tibetica*, *Bolbitis tonkinensis*, *Bolbitis umbrosa*, *Bolbitis vanuaensis*, and *Bolbitis virens*.

[0047] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Lomariopsidaceae*, Genus *Nephrolepis*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Lomariopsidaceae*, Genus *Nephrolepis* is selected from but not limited to *Nephrolepis abrupta*, *Nephrolepis acuminata*, *Nephrolepis acutifolia*, *Nephrolepis arida*, *Nephrolepis arthropteroides*, *Nephrolepis biserrata* var. *auriculata*, *Nephrolepis brownii*, *Nephrolepis celebica*, *Nephrolepis clementis*, *Nephrolepis cordifolia*, *Nephrolepis davalliae*, *Nephrolepis davallioides*, *Nephrolepis dayakorum*, *Nephrolepis delicatula*, *Nephrolepis dicksonioides*, *Nephrolepis duffii*, *Nephrolepis exaltata* ssp. *exaltata* ssp. *Hawaiiensis*, *Nephrolepis falcata*, *Nephrolepis falciformis*, *Nephrolepis glabra*, *Nephrolepis hirsutula*, *Nephrolepis humatoides*, *Nephrolepis iridescens*, *Nephrolepis kurotawae*, *Nephrolepis laurifolia*, *Nephrolepis lauterbachii*, *Nephrolepis lindsayae*, *Nephrolepis multifida*, *Nephrolepis multiflora*, *Nephrolepis niphoboloides*, *Nephrolepis obliterate*, *Nephrolepis paludosa*, *Nephrolepis pectinata*, *Nephrolepis pendula*, *Nephrolepis persicifolia*, *Nephrolepis pickelii*, *Nephrolepis pilosula*, *Nephrolepis pubescens*, *Nephrolepis pumicicola*, *Nephrolepis radicans*, *Nephrolepis rivularis*, *Nephrolepis rosenstockii*, *Nephrolepis saligna*, *Nephrolepis schlechteri*, *Nephrolepis serrate*, *Nephrolepis thomsoni*, *Nephrolepis undulata* var. *aureoglandulosa*, *Nephrolepis x averyi*., *Nephrolepis x copelandii*, and *Nephrolepis x medlerae*.

[0048] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae*, Genus *Campyloneurum*, Genus *Drynaria*, Genus *Lepisorus*, Genus *Microgramma*, Genus *Microsorium*, Genus *Neurodium*, Genus *Niphidium*, Genus *Pecluma* M.G., Genus *Phlebodium*,

Genus *Phymatosorus*, Genus *Platyserium*, Genus *Pleopeltis*, Genus *Polypodium* L.

[0049] In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Polypodium* L. In some aspects the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Polypodium* L. selected from but not limited to *Polypodium absidatum*, *Polypodium acutifolium*, *Polypodium adiantiforme*, *Polypodium aequale*, *Polypodium affine*, *Polypodium albidopaleatum*, *Polypodium alcornae*, *Polypodium alfarii*, *Polypodium alfredii*, *Polypodium alfredii* var. *curtii*, *Polypodium allosuroides*, *Polypodium alsophilicola*, *Polypodium amami-anum*, *Polypodium amoenum*, *Polypodium amorphum*, *Polypodium anetioides*, *Polypodium anfractuosum*, *Polypodium anguinum*, *Polypodium angustifolium* f. *remotifolia*, *Polypodium angustifolium* var. *amphostenon*, *Polypodium angustifolium* var. *heterolepis*, *Polypodium angustifolium* var. *monstrosa*, *Polypodium angustipaleatum*, *Polypodium angustissimum*, *Polypodium anisomeron* var. *pectinatum*, *Polypodium antioquianum*, *Polypodium aoristorum*, *Polypodium apagolepis*, *Polypodium apicidens*, *Polypodium apiculatum*, *Polypodium apoense*, *Polypodium appalachianum*, *Polypodium appressum*, *Polypodium arenarium*, *Polypodium argentinum*, *Polypodium argutum*, *Polypodium armatum*, *Polypodium aromaticum*, *Polypodium aspersum*, *Polypodium assurgens*, *Polypodium atrum*, *Polypodium auriculatum*, *Polypodium balaonense*, *Polypodium balliviani*, *Polypodium bamleri*, *Polypodium bangii*, *Polypodium bartlettii*, *Polypodium basale*, *Polypodium bernoullii*, *Polypodium biauratum*, *Polypodium bifrons*, *Polypodium blepharodes*, *Polypodium bolivari*, *Polypodium bolivianum*, *Polypodium bolobense*, *Polypodium bombycinum*, *Polypodium bombycinum* var. *insularum*, *Polypodium bradeorum*, *Polypodium bryophilum*, *Polypodium bryopodium*, *Polypodium buchtienii*, *Polypodium buesii*, *Polypodium bulbotrichum*, *Polypodium caceresii*, *Polypodium californicum* f. *brauscombii*, *Polypodium californicum* f. *parsonsiae*, *Polypodium californicum*, *Polypodium calophlebium*, *Polypodium calvum*, *Polypodium camptophyllum*, *Polypodium abbreviatum*, *Polypodium capitellatum*, *Polypodium carpintera*, *Polypodium chachapoyense*, *Polypodium chartaceum*, *Polypodium chimantense*, *Polypodium chiricanum*, *Polypodium choquetangense*, *Polypodium christensenii*, *Polypodium christii*, *Polypodium chrysotrichum*, *Polypodium ciliolepis*, *Polypodium cinerascens*, *Polypodium collinsii*, *Polypodium colysoides*, *Polypodium confluens*, *Polypodium conforme*, *Polypodium confusum*, *Polypodium congregatifolium*, *Polypodium connellii*, *Polypodium consimile* var. *bourgaeum*, *Polypodium consimile* var. *minor*, *Polypodium conterminans*, *Polypodium contiguum*, *Polypodium cookii*, *Polypodium coriaceum*, *Polypodium coronans*, *Polypodium costaricense*, *Polypodium costatum*, *Polypodium crassifolium* f. *angustissimum*, *Polypodium crassifolium* var. *longipes*, *Polypodium crassulum*, *Polypodium craterisorum*, *Polypodium cryptum*, *Polypodium crystalloneuron*, *Polypodium cucullatum* var. *planum*, *Polypodium cuencanum*, *Polypodium cumingianum*, *Polypodium cupreolepis*, *Polypodium curranii*, *Polypodium curvans*, *Polypodium cyathicola*, *Polypodium cyathisorum*, *Polypodium cyclocolpon*, *Polypodium daguense*, *Polypodium damunense*, *Polypodium dareiformioides*, *Polypodium dasyleura*, *Polypodium decipiens*, *Polypodium decorum*, *Polypodium delicatulum*, *Polypodium deltoideum*, *Polypodium demeraranum*, *Polypodium denticulatum*, *Polypodium diaphanum*, *Polypodium dilatatum*, *Polypodium dispersum*, *Polypodium dissectum*, *Polypodium dissimulans*, *Polypodium dolichosorum*, *Polypodium dolorensis*, *Polypodium donnell-smithii*, *Polypodium drymoglossoides*, *Polypodium ebeninum*, *Polypodium eggersii*, *Polypodium elmeri*, *Polypodium elongatum*, *Polypodium enterosoroides*, *Polypodium erubescens*, *Polypodium erythrolepis*, *Polypodium erythrotrichum*, *Polypodium eurybasis*, *Polypodium eurybasis* var. *villosum*, *Polypodium exornans*, *Polypodium falcoideum*, *Polypodium fallacissimum*, *Polypodium farinosum*, *Polypodium faucium*, *Polypodium feei*, *Polypodium ferrugineum*, *Polypodium feuillei*, *Polypodium firmulum*, *Polypodium firmum*, *Polypodium flaccidum*, *Polypodium flagellare*, *Polypodium flexuosum*, *Polypodium flexuosum* var. *ekmanii*, *Polypodium forbesii*, *Polypodium formosanum*, *Polypodium fraxinifolium* subsp. *articulatum*, *Polypodium fraxinifolium* subsp. *luridum*, *Polypodium fructuosum*, *Polypodium fucoides*, *Polypodium fulvescens*, *Polypodium galeottii*, *Polypodium glaucum*, *Polypodium glycyrrhiza*, *Polypodium gracillimum*, *Polypodium gramineum*, *Polypodium grandifolium*, *Polypodium gratum*, *Polypodium graveolens*, *Polypodium griseo-nigrum*, *Polypodium griseum*, *Polypodium guttatum*, *Polypodium haallioanum*, *Polypodium hammatisorum*, *Polypodium hancockii*, *Polypodium haplophlebicum*, *Polypodium harrisii*, *Polypodium hastatum* var. *simplex*, *Polypodium hawaiiense*, *Polypodium heanophyllum*, *Polypodium helleri*, *Polypodium hemionitidum*, *Polypodium henryi*, *Polypodium herzogii*, *Polypodium hesperium*, *Polypodium hessii*, *Polypodium hombersleyi*, *Polypodium hostmannii*, *Polypodium humile*, *Polypodium hyalinum*, *Polypodium iboense*, *Polypodium induens* var. *subdentatum*, *Polypodium insidiosum*, *Polypodium insigne*, *Polypodium intermedium* subsp. *masafueranum* var. *obtusesserratum*, *Polypodium intramarginale*, *Polypodium involutum*, *Polypodium itatiayense*, *Polypodium javanicum*, *Polypodium juglandifolium*, *Polypodium kaniense*, *Polypodium knowltoniorum*, *Polypodium kyimbilense*, *Polypodium l'herminieri* var. *costaricense*, *Polypodium lachniferum* f. *incurvata*, *Polypodium lachniferum* var. *glabrescens*, *Polypodium lachnopus*, *Polypodium lanceolatum* var. *complanatum*, *Polypodium lanceolatum* var. *trichophorum*, *Polypodium latevagans*, *Polypodium laxifrons*, *Polypodium laxifrons* var. *lividum*, *Polypodium lehmannianum*, *Polypodium leiorhizum*, *Polypodium leptopodon*, *Polypodium leuconeuron* var. *angustifolia*, *Polypodium leuconeuron* var. *latifolium*, *Polypodium leucosticta*, *Polypodium limulum*, *Polypodium lindigii*, *Polypodium lineatum*, *Polypodium lomarioides*, *Polypodium longifrons*, *Polypodium lorentense*, *Polypodium loriceum* var. *umbraticum*, *Polypodium loriforme*, *Polypodium loxogramme* f. *gigas*, *Polypodium ludens*, *Polypodium luzonicum*, *Polypodium lycopodioides* f. *obtusum*, *Polypodium lycopodioides* L., *Polypodium macrolepis*, *Polypodium macrophyllum*, *Polypodium macrosorum*, *Polypodium macrosphaerum*, *Polypodi-*

um maculosum, *Polypodium madrense*, *Polypodium manmeiense*, *Polypodium margaritiferum*, *Polypodium maritimum*,
Polypodium martensii, *Polypodium majoris*, *Polypodium megalolepis*, *Polypodium melanotrichum*, *Polypodium menis-*
ciifolium var. *pubescens*, *Polypodium meniscioides*, *Polypodium merrillii*, *Polypodium mettenii*, *Polypodium mexiae*,
5 *Polypodium microsorum*, *Polypodium militare*, *Polypodium minimum*, *Polypodium minusculum*, *Polypodium mixtum*,
Polypodium mollendense, *Polypodium mollissimum*, *Polypodium moniliforme* var. *minus*, *Polypodium monooides*, *Polyp-*
podium monticola, *Polypodium montigenum*, *Polypodium moritzianum*, *Polypodium moultonii*, *Polypodium multicauda-*
tum, *Polypodium multilineatum*, *Polypodium multisorum*, *Polypodium munchii*, *Polypodium muscoides*, *Polypodium myri-*
olepis, *Polypodium myriophyllum*, *Polypodium myriotrichum*, *Polypodium nematorhizon*, *Polypodium nemorale*, *Polyp-*
podium nesioticum, *Polypodium nigrescentium*, *Polypodium nigripes*, *Polypodium nigrocinctum*, *Polypodium nimbatum*,
10 *Polypodium nitidissimum*, *Polypodium nitidissimum* var. *latior*, *Polypodium nubrigenum*, *Polypodium oligolepis*, *Polypo-*
dium oligosorum, *Polypodium oligosorum*, *Polypodium olivaceum*, *Polypodium olivaceum* var. *elatum*, *Polypodium*
oodes, *Polypodium oosphaerum*, *Polypodium oreophilum*, *Polypodium ornatissimum*, *Polypodium ornatum*, *Polypodium*
ovatum, *Polypodium oxylobum*, *Polypodium oxypholis*, *Polypodium pakkaense*, *Polypodium pallidum*, *Polypodium pal-*
matopedatum, *Polypodium palmeri*, *Polypodium panamense*, *Polypodium parvum*, *Polypodium patagonicum*, *Polypo-*
15 *dium paucisorum*, *Polypodium pavonianum*, *Polypodium pectinatum* var. *caliense*, *Polypodium pectinatum* var. *hispidum*,
Polypodium pellucidum, *Polypodium pendulum* var. *boliviense*, *Polypodium percrassum*, *Polypodium perpusillum*, *Polyp-*
odium peruvianum var. *subgibbosum*, *Polypodium phyllitidis* var. *elongatum*, *Polypodium pichinchense*, *Polypodium*
pilosissimum, *Polypodium pilosissimum* var. *glabriusculum*, *Polypodium pilosissimum* var. *tunguraquensis*, *Polypodium*
pityrolepis, *Polypodium platyphyllum*, *Polypodium playfairii*, *Polypodium plebeium* var. *cooperi*, *Polypodium plectolepid-*
20 *ioides*, *Polypodium pleolepis*, *Polypodium plesiosorum* var. *i.*, *Polypodium podobasis*, *Polypodium podocarpum*, *Polypo-*
dium poloense, *Polypodium polydatylon*, *Polypodium polypodioides* var. *aciculare*, *Polypodium polypodioides* var.
michauxianum, *Polypodium praetermissum*, *Polypodium preslianum* var. *immersum*, *Polypodium procerum*, *Polypodium*
procerum, *Polypodium productum*, *Polypodium productum*, *Polypodium prolongilobum*, *Polypodium propinguum*, *Polyp-*
odium proteus, *Polypodium pruinatum*, *Polypodium pseudocapillare*, *Polypodium pseudofraternum*, *Polypodium pseu-*
25 *donutans*, *Polypodium pseudoserratum*, *Polypodium pulcherrimum*, *Polypodium pulogense*, *Polypodium pungens*, *Polyp-*
odium purpusii, *Polypodium radicale*, *Polypodium randallii*, *Polypodium ratiborii*, *Polypodium reclinatum*, *Polypodium*
recreense, *Polypodium repens* var. *abruptum*, *Polypodium revolvens*, *Polypodium rhachipterygium*, *Polypodium rhom-*
boideum, *Polypodium rigens*, *Polypodium robustum*, *Polypodium roraimense*, *Polypodium roraimense*, *Polypodium ro-*
sei, *Polypodium rosenstockii*, *Polypodium rubidum*, *Polypodium rudimentum*, *Polypodium rusbyi*, *Polypodium sablani-*
30 *anum*, *Polypodium sarmentosum*, *Polypodium saxicola*, *Polypodium schenckii*, *Polypodium schlechteri*, *Polypodium*
scolopendria, *Polypodium scolopendria*, *Polypodium scolopendrium*, *Polypodium scouleri*, *Polypodium scutulatum*, *Polyp-*
odium segregatum, *Polypodium semihirsutum*, *Polypodium semihirsutum* var. *fuscsetosum*, *Polypodium senile* var.
minor, *Polypodium sericeolanatum*, *Polypodium serraeforme*, *Polypodium serricula*, *Polypodium sesquipedala*, *Polypo-*
35 *dium sessilifolium*, *Polypodium setosum* var. *calvum*, *Polypodium setulosum*, *Polypodium shaferi*, *Polypodium sibo-*
mense, *Polypodium siccum*, *Polypodium simacense*, *Polypodium simulans*, *Polypodium singeri*, *Polypodium sinicum*,
Polypodium sintensisii, *Polypodium skutchii*, *Polypodium sloanei*, *Polypodium sodiroi*, *Polypodium sordidulum*, *Polypo-*
dium sordidum, *Polypodium sphaeropteroides*, *Polypodium sphenodes*, *Polypodium sprucei*, *Polypodium sprucei* var.
furcativenosa, *Polypodium steirolepis*, *Polypodium stenobasis*, *Polypodium stenolepis*, *Polypodium stenopterum*, *Polyp-*
40 *odium subcapillare*, *Polypodium subflabelliforme*, *Polypodium subhemionitidium*, *Polypodium subinaequale*, *Polypo-*
dium subintegrum, *Polypodium subspathulatum*, *Polypodium subtile*, *Polypodium subvestitum*, *Polypodium subviride*,
Polypodium superficiale var. *attenuatum*, *Polypodium superficiale* var. *chinensis*, *Polypodium sursumcurrens*, *Polypo-*
dium tablazianum, *Polypodium taenifolium*, *Polypodium tamandarei*, *Polypodium tatei*, *Polypodium tenuiculum* var.
acrosora, *Polypodium tenuiculum* var. *brasiliense*, *Polypodium tenuilore*, *Polypodium tenuinerve*, *Polypodium tepuiense*,
45 *Polypodium teresae*, *Polypodium tetragonum* var. *incompletum*, *Polypodium thysanolepis* var. *bipinnatifidum*, *Polypo-*
dium thysanolepis, var. *thysanolepis*, *Polypodium thysanolepsi*, *Polypodium tobagense*, *Polypodium trichophyllum*,
Polypodium tridactylum, *Polypodium tridentatum*, *Polypodium trifurcatum* var. *brevipes*, *Polypodium triglossum*, *Polyp-*
odium truncatulum, *Polypodium truncicola* var. *major*, *Polypodium truncicola* var. *minor*, *Polypodium tuberosum*, *Polyp-*
50 *odium tunguraguae*, *Polypodium turquinum*, *Polypodium turrialbae*, *Polypodium ursipes*, *Polypodium vagans*, *Polypo-*
dium valdealatum, *Polypodium versteegii*, *Polypodium villagrani*, *Polypodium virginianum* f. *cambroideum*, *Polypodium*
virginianum f. *periferens*, *Polypodium vittarioides*, *Polypodium vulgare*, *Polypodium vulgare* L., *Polypodium vulgare*
subsp. oreophilum, *Polypodium vulgare* var. *acuminatum*, *Polypodium vulpinum*, *Polypodium williamsii*, *Polypodium*
wobbense, *Polypodium x fallacissimum-guttatum*, *Polypodium xantholepis*, *Polypodium xiphopteris*, *Polypodium yaru-*
malense, *Polypodium yungense*, and *Polypodium zosteriforme*.

[0050] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in
55 the Order Polypodiales, Family Polypodiaceae, Genus *Platycterium*. In some aspects the nucleic acid molecule encoding
the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platyc-*
terium selected from but not limited to *Platycterium alcicorne*, *Platycterium andinum*, *Platycterium angolense*, *Platycterium*
bifurcatum, *Platycterium coronarium*, *Platycterium elephantotis*, *Platycterium ellisii*, *Platycterium grande*, *Platycterium hillii*,

Platycterium holttumii, *Platycterium madagascariense*, *Platycterium quadridichotomum*, *Platycterium ridleyi*, *Platycterium* sp. ES-2011, *Platycterium stemaria*, *Platycterium superbum*, *Platycterium veitchii*, *Platycterium wallichii*, *Platycterium wandae*, *Platycterium wilhelminaereginae*, and *Platycterium willinckii*.

[0051] In some embodiments the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Division *Lycophyta*. The phylogeny of extant Lycopods as used herein is based on the classification by N. Wikstrom, American Fern Journal, 91:150-156 (2001). Other phylogenetic classifications of extant Lycopods are known to one skilled in the art. Additional information on the phylogeny of ferns can be found at mobot.org/MOBOT/research/APweb/ (which can be accessed using the "www" prefix) and Schuettpelz E. and Pryer K. M., TAXON 56: 1037-1050 (2007) based on three plastid genes. Additional Lycopod species can be found at homepages.caverock.net.nz/~bj/fern/list.htm (which can be accessed using the http:// prefix).

[0052] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Class *Isoetopsida* or Class *Lycopodiopsida*.

[0053] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Class *Isoetopsida* Order *Selaginales*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Class *Isoetopsida*, Order *Selaginales*, Family *Selaginellaceae*. In some embodiments the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Genus *Selaginella*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a *Selaginella* species selected from but not limited to *Selaginella acanthonota*, *Selaginella apoda*, *Selaginella arbuscula*, *Selaginella arenicola*, *Selaginella arizonica*, *Selaginella armata*, *Selaginella asprella*, *Selaginella bififormis*, *Selaginella bigelovii*, *Selaginella braunii*, *Selaginella cinerascens*, *Selaginella cordifolia*, *Selaginella deflexa*, *Selaginella delicatula*, *Selaginella densa*, *Selaginella douglasii*, *Selaginella eatonii*, *Selaginella eclipses*, *Selaginella eremophila*, *Selaginella erythropus*, *Selaginella flabellata*, *Selaginella hansenii*, *Selaginella heterodonta*, *Selaginella kraussiana*, *Selaginella krugii*, *Selaginella laxifolia*, *Selaginella lepidophylla*, *Selaginella leucobryoides*, *Selaginella ludoviciana*, *Selaginella mutica*, *Selaginella oregana*, *Selaginella ovifolia*, *Selaginella pallescens*, *Selaginella peruviana*, *Selaginella pilifera*, *Selaginella plana*, *Selaginella plumosa*, *Selaginella pulcherrima*, *Selaginella rupestris*, *Selaginella rupincola*, *Selaginella scopulorum*, *Selaginella selaginoides*, *Selaginella sibirica*, *Selaginella standleyi*, *Selaginella stellata*, *Selaginella subcaulescens*, *Selaginella substipitata*, *Selaginella tenella*, *Selaginella tortipila*, *Selaginella uliginosa*, *Selaginella umbrosa*, *Selaginella uncinata*, *Selaginella underwoodii*, *Selaginella utahensis*, *Selaginella victoriae*, *Selaginella viridissima*, *Selaginella wallacei*, *Selaginella watsonii*, *Selaginella weatherbiana*, *Selaginella willdenowii*, *Selaginella wrightii* and *Selaginella X neomexicana*.

[0054] In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Class *Lycopodiopsida*, Order *Lycopodiales*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a fern species in the Class *Lycopodiopsida*, Order *Lycopodiales* Family *Lycopodiaceae* or Family *Huperziaceae*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Genus *Austrolycopodium*, *Dendrolycopodium*, *Diphasiastrum*, *Diphasium*, *Huperzia*, *Lateristachys*, *Lycopodiastrum*, *Lycopodiella*, *Lycopodium*, *Palhinhaea*, *Pseudodiphasium*, *Pseudolycopodiella*, *Pseudolycopodium* or *Spinulum*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Genus *Lycopodium*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a *Lycopodium* species selected from but not limited to *Lycopodium alpinum* L., *Lycopodium annotinum* L., *Lycopodium clavatum* L., *Lycopodium complanatum* L., *Lycopodium dendroideum* Michx., *Lycopodium digitatum*, *Lycopodium xhabereri*, *Lycopodium hickeyi*, *Lycopodium xissleri*, *Lycopodium lagopus*, *Lycopodium obscurum* L., *Lycopodium phlegmaria* L., *Lycopodium sabinifolium*, *Lycopodium sitchense*, *Lycopodium tristachyum*, *Lycopodium venustulum*, *Lycopodium venustulum* var. *venustulum*, *Lycopodium venustulum* var. *verticale*, *Lycopodium volubile* and *Lycopodium xzeilleri*.

[0055] In some embodiments the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species in the Genus *Huperzia*. In some aspects the nucleic acid molecule encoding the PtlP-50 polypeptide is derived from a species selected from but not limited to *Huperzia appressa*, *Huperzia arctica*, *Huperzia attenuata*, *Huperzia australiana*, *Huperzia balansae*, *Huperzia billardierei*, *Huperzia brassii*, *Huperzia campiana*, *Huperzia capellae*, *Huperzia carinata*, *Huperzia cf. carinata* ARF000603, *Huperzia cf. nummulariifolia* ARF001140, *Huperzia cf. phlegmaria* ARF000717, *Huperzia cf. phlegmaria* ARF000771, *Huperzia cf. phlegmaria* ARF000785, *Huperzia cf. phlegmaria* ARF001007, *Huperzia cf. phlegmaria* ARF002568, *Huperzia cf. phlegmaria* ARF002703, *Huperzia cf. phlegmaria* Wikstrom 1998, *Huperzia chinensis*, *Huperzia compacta*, *Huperzia crassa*, *Huperzia crispata*, *Huperzia cryptomeriana*, *Huperzia cumingii*, *Huperzia dacrydioides*, *Huperzia dalhousieana*, *Huperzia dichotoma*, *Huperzia emeiensis*, *Huperzia ericifolia*, *Huperzia eversa*, *Huperzia fargesii*, *Huperzia fordii*, *Huperzia funiformis*, *Huperzia goebellii*, *Huperzia haleakalae*, *Huperzia hamiltonii*, *Huperzia heteroclita*, *Huperzia hippuridea*, *Huperzia hippuris*, *Huperzia holstii*, *Huperzia horizontalis*, *Huperzia hunanensis*, *Huperzia hystrix*, *Huperzia lindenbergii*, *Huperzia linifolia*, *Huperzia lockyeri*, *Huperzia lucidula*, *Huperzia mingcheensis*, *Huperzia miyoshiana*, *Huperzia nanchuanensis*, *Huperzia nummulariifolia*, *Huperzia obtusifolia*, *Huperzia ophioglossoides*, *Huperzia petiolata*, *Huperzia phlegmaria*, *Huperzia phlegmarioides*, *Huperzia phyllantha*, *Huperzia pinifolia*, *Huperzia polydactyla*, *Huperzia prolifera*, *Huperzia reflexa*, *Huperzia rosenstockiana*, *Huperzia rufescens*, *Huperzia salvinoides*, *Huperzia sarmentosa*, *Huperzia selago*, *Huperzia serrata*, *Huperzia sieboldii*, *Huperzia somae*, *Huperzia*

squarrosa, *Huperzia subulata*, *Huperzia sutchueniana*, *Huperzia tauri*, *Huperzia taxifolia*, *Huperzia tenuis*, *Huperzia tetragona*, *Huperzia tetrasticha*, *Huperzia unguiculata*, *Huperzia varia*, *Huperzia verticillata* and *Huperzia wilsonii*.

Polynucleotides encoding PtIP-65 polypeptides

5

[0056] One source of polynucleotides that encode PtIP-65 polypeptides or related proteins is a fern or other primitive plant species which contains a PtIP-65 polynucleotide encoding a PtIP-65 polypeptide. The polynucleotides can be used to express PtIP-65 polypeptides in bacterial hosts that include but are not limited to *Agrobacterium*, *Bacillus*, *Escherichia*, *Salmonella*, *Pseudomonas* and *Rhizobium* bacterial host cells. The polynucleotides are also useful as probes for isolating homologous or substantially homologous polynucleotides that encode PtIP-65 polypeptides or related proteins. Such probes can be used to identify homologous or substantially homologous polynucleotides derived from *Pteridophyta* species.

10

[0057] Polynucleotides that encode PtIP-65 polypeptides can also be synthesized de novo from a PtIP-65 polypeptide sequence. The sequence of the polynucleotide gene can be deduced from a PtIP-65 polypeptide sequence through use of the genetic code. Computer programs such as "BackTranslate" (GCG™ Package, Acclerys, Inc. San Diego, Calif.) can be used to convert a peptide sequence to the corresponding nucleotide sequence encoding the peptide. Furthermore, synthetic PtIP-65 polynucleotide sequences of the disclosure can be designed so that they will be expressed in plants. US Patent Number 5,500,365 describes a method for synthesizing plant genes to improve the expression level of the protein encoded by the synthesized gene. This method relates to the modification of the structural gene sequences of the exogenous transgene, to cause them to be more efficiently transcribed, processed, translated and expressed by the plant. Features of genes that are expressed well in plants include elimination of sequences that can cause undesired intron splicing or polyadenylation in the coding region of a gene transcript while retaining substantially the amino acid sequence of the toxic portion of the insecticidal protein. A similar method for obtaining enhanced expression of transgenes in monocotyledonous plants is disclosed in US Patent Number 5,689,052.

15

20

[0058] "Complement" is used herein to refer to a nucleic acid sequence that is sufficiently complementary to a given nucleic acid sequence such that it can hybridize to the given nucleic acid sequence to thereby form a stable duplex. "Polynucleotide sequence variants" is used herein to refer to a nucleic acid sequence that except for the degeneracy of the genetic code encodes the same polypeptide.

25

[0059] In some aspects the nucleic acid molecule encoding the PtIP-65 polypeptide is a non-genomic nucleic acid sequence. As used herein a "non-genomic nucleic acid sequence" or "non-genomic nucleic acid molecule" or "non-genomic polynucleotide" refers to a nucleic acid molecule that has one or more change in the nucleic acid sequence compared to a native or genomic nucleic acid sequence. In some aspects the change to a native or genomic nucleic acid molecule includes but is not limited to: changes in the nucleic acid sequence due to the degeneracy of the genetic code; codon optimization of the nucleic acid sequence for expression in plants; changes in the nucleic acid sequence to introduce at least one amino acid substitution, insertion, deletion and/or addition compared to the native or genomic sequence; removal of one or more intron associated with the genomic nucleic acid sequence; insertion of one or more heterologous introns; deletion of one or more upstream or downstream regulatory regions associated with the genomic nucleic acid sequence; insertion of one or more heterologous upstream or downstream regulatory regions; deletion of the 5' and/or 3' untranslated region associated with the genomic nucleic acid sequence; insertion of a heterologous 5' and/or 3' untranslated region; and modification of a polyadenylation site. In some aspects the non-genomic nucleic acid molecule is a cDNA. In some aspects the non-genomic nucleic acid molecule is a synthetic nucleic acid sequence.

30

35

40

[0060] In some embodiments the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Division *Pteridophyta*. The phylogeny of ferns as used herein is based on the classification for extant ferns by A. R. Smith et al, TAXON, 55:705-731 (2006). The consensus phylogeny based on the classification by A. R. Smith is shown in Figure 1. Additional information on the phylogeny of ferns can be found at mobot.org/MOBOT/research/APweb/ (which can be accessed using the "www" prefix) and Schuettpelz E. and Pryer K. M., TAXON 56: 1037-1050 (2007) based on three plastid genes. Additional fern and other primitive plant species can be found at homepages.caverock.net.nz/~bj/fern/list.htm (which can be accessed using the http:// prefix).

45

[0061] In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Psilotales*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*, Family *Psilotaceae*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales* Family *Ophioglossaceae*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Genus *Ophioglossum* L., *Botrychium*, *Botrypus*, *Helminthostachys*, *Ophioderma*, *Cheiroglossa*, *Sceptridium* or *Mankyua*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the *Ophioglossum* L. Genus is selected from

50

55

but not limited to *Ophioglossum californicum*, *Ophioglossum coriaceum*, *Ophioglossum costatum*, *Ophioglossum cro-talophoroides*, *Ophioglossum engelmannii*, *Ophioglossum falcatum*, *Ophioglossum gomezianum*, *Ophioglossum grami-neum*, *Ophioglossum kawamurae*, *Ophioglossum lusitanicum*, *Ophioglossum namegatae*, *Ophioglossum nudicaule*, *Ophioglossum palmatum*, *Ophioglossum parvum*, *Ophioglossum pedunculatum*, *Ophioglossum pendulum*, *Ophioglos-sum petiolatum*, *Ophioglossum pusillum*, *Ophioglossum reticulatum*, *Ophioglossum richardsiae*, *Ophioglossum ther-male*, and *Ophioglossum vulgatum*.

[0062] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Class *Polypodiopsida/Pteridopsida*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Osmundales* (royal ferns); Family *Osmundaceae*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Hymenophyllales* (filmy ferns and bristle ferns); Family *Hymenophyllaceae*. In some embodiments the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Gleicheniales*; Family *Gleicheniaceae*, Family *Dipteridaceae* or Family *Matoniaceae*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Schizaeales*; Family *Lygodiaceae*, Family *Anemiaceae* or Family *Schizaeaceae*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Salviniales*; Family *Marsileaceae* or Family *Salviniaceae*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Cyatheales*; Family *Thyrsopteridaceae*, Family *Loxsomataceae*, Family *Culcitaceae*, Family *Plagiogyriaceae*, Family *Cibotiaceae*, Family *Cyatheaceae*, Family *Dicksoniaceae* or Family

Metaxyaceae.

[0063] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Polypodiales*; Family *Lindsaeaceae*, Family *Saccolomataceae*, Family *Cystodiaceae*, Family *Dennstaedtiaceae*, Family *Pteridaceae*, Family *Aspleniaceae*, Family *Thelypteridaceae*, Family *Woodsiaceae*, Family *Onocleaceae*, Family *Blechnaceae*, Family *Dryopteridaceae*, Family *Lomariopsidaceae*, Family *Tectariaceae*, Family *Oleandraceae*, Family *Davalliaceae* or Family *Polypodiaceae*.

[0064] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Polypodiales*, Family *Pteridaceae*, Genus *Adiantaceae* selected from but not limited to *Adiantum aethiopicum*, *Adiantum aleuticum*, *Adiantum bonatianum*, *Adiantum cajennense*, *Adiantum capillus-junonis*, *Adiantum capillus-veneris*, *Adiantum caudatum*, *Adiantum chienii*, *Adiantum chilense*, *Adiantum cuneatum*, *Adiantum cunninghamii*, *Adiantum davidii*, *Adiantum diaphanum*, *Adiantum edentulum*, *Adiantum edgeworthii*, *Adiantum excisum*, *Adiantum feng-ianum*, *Adiantum fimbriatum*, *Adiantum flabellulatum*, *Adiantum formosanum*, *Adiantum formosum*, *Adiantum fulvum*, *Adiantum gravesii*, *Adiantum hispidulum*, *Adiantum induratum*, *Adiantum jordanii*, *Adiantum juxtapositum*, *Adiantum latifolium*, *Adiantum leveillei*, *Adiantum lianxianense*, *Adiantum malesianum*, *Adiantum mariesii*, *Adiantum monochlamys*, *Adiantum myriosorum*, *Adiantum obliquum*, *Adiantum ogasawarensense*, *Adiantum pedatum*, *Adiantum pentadactylon*, *Adiantum peruvianum*, *Adiantum philippense*, *Adiantum princeps*, *Adiantum pubescens*, *Adiantum raddianum*, *Adiantum reniforme*, *Adiantum roborowskii*, *Adiantum serratodentatum*, *Adiantum sinicum*, *Adiantum soboliferum*, *Adiantum sub-cordatum*, *Adiantum tenerum*, *Adiantum terminatum*, *Adiantum tetraphyllum*, *Adiantum venustum*, *Adiantum viridescens*, and *Adiantum viridimontanum*.

[0065] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Polypodiales*, Family *Aspleniaceae*, Genus *Asplenium L.* In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Order *Polypodiales*, Family *Aspleniaceae*, Genus *Asplenium L.* selected from but not limited to *Asplenium abbreviatum*, *Asplenium abrotanoides*, *Asplenium abscissum* var. *sub-aequilaterale*, *Asplenium abscissum*, *Asplenium achilleifolium*, *Asplenium acuminatum*, *Asplenium adiantifrons*, *Asple-nium adiantoides*, *Asplenium adiantoides* var. *squamulosum*, *Asplenium adiantum-nigrum L.*, *Asplenium adiantum-nigrum* var. *adiantum-nigrum*, *Asplenium adiantum-nigrum* var. *yuanum*, *Asplenium adnatum*, *Asplenium aethiopicum*, *Asplenium affine*, *Asplenium affine* var. *affine*, *Asplenium affine* var. *gilpinae*, *Asplenium affine* var. *mettenii*, *Asplenium affine* var. *pecten*, *Asplenium africanum*, *Asplenium afzelii*, *Asplenium aitchisonii*, *Asplenium alatulum*, *Asplenium alatum*, *Asplenium alfredii*, *Asplenium altajense*, *Asplenium amabile*, *Asplenium ambohitantelense*, *Asplenium anceps* var. *pro-liferum*, *Asplenium andapense*, *Asplenium andersonii*, *Asplenium angustatum*, *Asplenium angustum*, *Asplenium anis-ophyllum*, *Asplenium annetii*, *Asplenium antiquum*, *Asplenium antrophyoides*, *Asplenium apertum*, *Asplenium apoga-mum*, *Asplenium aquaticum*, *Asplenium arboreum*, *Asplenium arcanum*, *Asplenium arcuatum*, *Asplenium argentinum*, *Asplenium argutum*, *Asplenium aspidiiforme*, *Asplenium aspidioides*, *Asplenium asterolepis*, *Asplenium auricularium* var. *acutidens*, *Asplenium auricularium* var. *subintegerrimum*, *Asplenium auriculatum*, *Asplenium auriculatum* var. *aequi-laterale*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *nana*, *Asplenium auritum*, *Asplenium auritum* var. *auriculatum*, *Asplenium auritum* var. *auritum*, *Asplenium auritum* var. *bipinnatifidum*, *Asplenium auritum* var. *bipinnatisectum*, *Asplenium auritum* var. *davallioides*, *Asplenium auritum* var. *macilentum*, *As-plenium auritum* var. *rigidum*, *Asplenium auritum* var. *subsimplex*, *Asplenium austrochinense*, *Asplenium ayopayense*,

Asplenium badinii, *Asplenium balense*, *Asplenium ballivianii*, *Asplenium bangii*, *Asplenium bangii*, *Asplenium barbaense*,
Asplenium barclayanum, *Asplenium barkamense*, *Asplenium barteri*, *Asplenium basispicum*, *Asplenium bicrenatum*,
Asplenium bifrons, *Asplenium bipartitum*, *Asplenium blastophorum*, *Asplenium blepharodes*, *Asplenium blepharopho-*
5 *rum*, *Asplenium boiteaui*, *Asplenium bolivianum*, *Asplenium boltonii*, *Asplenium borealichinense*, *Asplenium bradei*,
Asplenium bradeorum, *Asplenium bradleyi*, *Asplenium brausei*, *Asplenium breedlovei*, *Asplenium buettneri*, *Asplenium*
buettneri var. *hildebrandtii*, *Asplenium bulbiferum*, *Asplenium bullatum* var. *bullatum*, *Asplenium bullatum* var. *shikoki-*
anum, *Asplenium bullatum*, *Asplenium cancellatum*, *Asplenium capillipes*, *Asplenium cardiophyllum* (Hance), *Asplenium*
caripense, *Asplenium carvalhoanum*, *Asplenium castaneoviride*, *Asplenium castaneum*, *Asplenium caudatum*, *Asple-*
10 *nium celtidifolium* (Kunze), *Asplenium ceratolepis*, *Asplenium changputungense*, *Asplenium chaseanum*, *Asplenium*
cheilosorum, *Asplenium chengkouense*, *Asplenium chihuahuense*, *Asplenium chimantae*, *Asplenium chimborazense*,
Asplenium chingianum, *Asplenium chlorophyllum*, *Asplenium chondrophyllum*, *Asplenium cicutarium*, *Asplenium cicu-*
tarium var. *paleaceum*, *Asplenium cirrhatum*, *Asplenium cladolepton*, *Asplenium clausenii*, *Asplenium coenobiale*,
Asplenium commutatum, *Asplenium congestum*, *Asplenium conquisitum*, *Asplenium consimile*, *Asplenium contiguum*,
Asplenium contiguum var. *hirtulum*, *Asplenium corderoi*, *Asplenium cordovense*, *Asplenium coriaceum*, *Asplenium cori-*
15 *ifolium*, *Asplenium correardii*, *Asplenium costale*, *Asplenium costale* var. *robustum*, *Asplenium cowanii*, *Asplenium crenu-*
latoserrulatum, *Asplenium crenulatum*, *Asplenium crinicaule*, *Asplenium crinulosum*, *Asplenium cristatum*, *Asplenium*
cryptolepis Fernald, *Asplenium cultrifolium* L., *Asplenium cuneatiforme*, *Asplenium cuneatum*, *Asplenium curvatum*,
Asplenium cuspidatum, *Asplenium cuspidatum* var. *cuspidatum*, *Asplenium cuspidatum* var. *foeniculaceum*, *Asplenium*
cuspidatum var. *triculum*, *Asplenium cuspidatum* var. *tripinnatum*, *Asplenium dalhousiae*, *Asplenium dareoides*, *Asple-*
20 *nium davallioides*, *Asplenium davisii*, *Asplenium debile*, *Asplenium debile*, *Asplenium decussatum*, *Asplenium delavayi*,
Asplenium delicatulum, *Asplenium delicatulum* var. *cocosensis*, *Asplenium delitescens*, *Asplenium delitescens* X *laetum*,
Asplenium densum, *Asplenium dentatum* L., *Asplenium dentatum* L., *Asplenium depauperatum*, *Asplenium deqenense*,
Asplenium diana, *Asplenium difforme*, *Asplenium dilatatum*, *Asplenium dimidiatum*, *Asplenium dimidiatum* var. *bolivi-*
25 *ense*, *Asplenium diplazisorum*, *Asplenium dissectum*, *Asplenium distans*, *Asplenium divaricatum*, *Asplenium divergens*,
Asplenium divisissimum, *Asplenium doederleinii*, *Asplenium donnell-smithii*, *Asplenium dregeanum*, *Asplenium du-*
longjiangense, *Asplenium duplicatoserratum*, *Asplenium eatonii*, *Asplenium ebeneum*, *Asplenium ebenoides*, *Asplenium*
ecuadoreense, *Asplenium eggersii*, *Asplenium emarginatum*, *Asplenium enatum*, *Asplenium ensiforme* fo. *bicuspe*, *As-*
plenium ensiforme fo. *ensiforme*, *Asplenium ensiforme* fo. *stenophyllum*, *Asplenium ensiforme*, *Asplenium erectum* var.
erectum, *Asplenium erectum* var. *gracile*, *Asplenium erectum* var. *usambarense*, *Asplenium erectum* var. *zeyheri*, &
30 *Asplenium erosum* L., *Asplenium escaleroense*, *Asplenium esculentum*, *Asplenium eutecnum*, *Asplenium excelsum*,
Asplenium excisum, *Asplenium exiguum*, *Asplenium extensum*, *Asplenium falcatum*, *Asplenium falcinellum*, *Asplenium*
faurei, *Asplenium feei*, *Asplenium fengyangshanense*, *Asplenium ferulaceum*, *Asplenium fibrillosum*, *Asplenium filix-*
femina, *Asplenium finckii*, *Asplenium finlaysonianum*, *Asplenium flabellulatum*, *Asplenium flabellulatum* var. *flabellulatum*,
Asplenium flabellulatum var. *partitum*, *Asplenium flaccidum*, *Asplenium flavescens*, *Asplenium flavidum*, *Asplenium*
35 *flexuosum*, *Asplenium fluminense*, *Asplenium foeniculaceum*, *Asplenium formosanum*, *Asplenium formosum* var. *caro-*
linum, *Asplenium formosum* var. *incultum*, *Asplenium formosum*, *Asplenium fournieri*, *Asplenium fragile*, *Asplenium*
fragile var. *lomense*, *Asplenium fragrans*, *Asplenium fragrans* var. *foeniculaceum*, *Asplenium franconis* var. *gracile*,
Asplenium fraxinifolium, *Asplenium friesiorum*, *Asplenium friesiorum* var. *nesophilum*, *Asplenium fugax*, *Asplenium fu-*
40 *jianense*, *Asplenium furcatum*, *Asplenium furfuraceum*, *Asplenium fuscipes*, *Asplenium fuscopubescens*, *Asplenium*
galeottii, *Asplenium gautieri*, *Asplenium gemmiferum*, *Asplenium gentryi*, *Asplenium geppii*, *Asplenium ghiesbreghtii*,
Asplenium gilliesii, *Asplenium gilpinae*, *Asplenium glanduliserratum*, *Asplenium glenniei*, *Asplenium goldmannii*, *Asple-*
nium gomezianum, *Asplenium grande*, *Asplenium grandifolium*, *Asplenium grandifrons*, *Asplenium gregoriae*, *Asplenium*
griffithianum, *Asplenium gulingense*, *Asplenium hainanense*, *Asplenium hallbergii*, *Asplenium hallei*, *Asplenium hallii*,
45 *Asplenium hangzhouense*, *Asplenium haplophyllum*, *Asplenium harpeodes*, *Asplenium harpeodes* var. *glaucovirans*,
Asplenium harpeodes var. *incisum*, *Asplenium harrisii* Jenman, *Asplenium harrisonii*, *Asplenium hastatum*, *Asplenium*
hebeiense, *Asplenium hemionitideum*, *Asplenium hemitomum*, *Asplenium henryi*, *Asplenium herpetopteris*, *Asplenium*
herpetopteris var. *herpetopteris*, *Asplenium herpetopteris* var. *acutipinnata*, *Asplenium herpetopteris* var. *masoulae*,
Asplenium herpetopteris var. *villosum*, *Asplenium hesperium*, *Asplenium heterochroum*, *Asplenium hians*, *Asplenium*
50 *hians* var. *palescens*, *Asplenium hoffmannii*, *Asplenium holophlebium*, *Asplenium hondoense*, *Asplenium horridum*,
Asplenium hostmannii, *Asplenium humistratum*, *Asplenium hypomelas*, *Asplenium inaequilaterale*, *Asplenium incisum*,
Asplenium incurvatum, *Asplenium indicum*, *Asplenium indicum* var. *indicum*, *Asplenium indicum* var. *yoshingagae*,
Asplenium induratum, *Asplenium indusiatum*, *Asplenium inexpectatum*, *Asplenium insigne*, *Asplenium insiticium*, *As-*
plenium insolitum, *Asplenium integerrimum*, *Asplenium interjectum*, *Asplenium jamesonii*, *Asplenium jaundeense*, *As-*
55 *plenium juglandifolium*, *Asplenium kangdingense*, *Asplenium kansuense*, *Asplenium kassneri*, *Asplenium kaulfussii*,
Asplenium kellermanii, *Asplenium kentuckiense*, *Asplenium khullarii*, *Asplenium kiangsuense*, *Asplenium kunzeanum*,
Asplenium lacerum, *Asplenium laciniatum*, *Asplenium laciniatum* var. *acutipinna*, *Asplenium laciniatum* var. *laciniatum*,
Asplenium laetum fo. *minor*, *Asplenium laetum*, *Asplenium laetum* var. *incisoserratum*, *Asplenium lamprocaulon*, *As-*
plenium laserpitiifolium var. *morrisonense*, *Asplenium lastii*, *Asplenium latedens*, *Asplenium latifolium*, *Asplenium laui*,

Asplenium laurentii, *Asplenium leandrianum*, *Asplenium lechleri*, *Asplenium leiboense*, *Asplenium lepidorachis*, *Asplenium leptochlamys*, *Asplenium leptophyllum*, *Asplenium levyi*, *Asplenium lindbergii*, *Asplenium lindenii*, *Asplenium lineatum*, *Asplenium lividum*, *Asplenium lobatum*, *Asplenium lobulatum*, *Asplenium lokohoense*, *Asplenium longicauda*, *Asplenium longicaudatum*, *Asplenium longifolium*, *Asplenium longisorum*, *Asplenium longjinense*, *Asplenium lorentzii*,
5 *Asplenium loriceum*, *Asplenium loxogrammoides*, *Asplenium lugubre*, *Asplenium lunulatum*, *Asplenium lunulatum* var. *pteropus*, *Asplenium lushanense*, *Asplenium lydgatei*, *Asplenium macilentum*, *Asplenium macraei*, *Asplenium macrodictyon*, *Asplenium macrophlebium*, *Asplenium macrophyllum*, *Asplenium macropterum*, *Asplenium macrosorum*, *Asplenium macrotis*, *Asplenium macrurum*, *Asplenium mainlingense*, *Asplenium mangindranense*, *Asplenium mannii*, *Asplenium marginatum* L., *Asplenium marojejense*, *Asplenium martianum*, *Asplenium matsumurae*, *Asplenium mauritiensis* Lorence, *Asplenium maximum*, *Asplenium*, ii, *Asplenium megalura*, *Asplenium megaphyllum*, *Asplenium meiotomum*, *Asplenium melanopus*, *Asplenium membranifolium*, *Asplenium meniscioides*, *Asplenium mesosorum*, *Asplenium mexicanum*, *Asplenium micropaleatum*, *Asplenium microtum*, *Asplenium mildbraedii*, *Asplenium mildei*, *Asplenium minimum*, *Asplenium minutum*, *Asplenium miradoreense*, *Asplenium miyunense*, *Asplenium mocceanum*, *Asplenium mocquersii*, *Asplenium modestum*, *Asplenium monanthes* var. *menziesii*, *Asplenium monanthes* L., *Asplenium monanthes* var. *monanthes*, *Asplenium monanthes* var. *castaneum*, *Asplenium monanthes* var. *wagneri*, *Asplenium monanthes* var. *yungense*, *Asplenium monodon*, *Asplenium montanum*, *Asplenium mosetenense*, *Asplenium moupinense*, *Asplenium mucronatum*, *Asplenium munchii*, *Asplenium muticum*, *Asplenium myapteron*, *Asplenium myriophyllum*, *Asplenium nakanoanum*, *Asplenium nanchuanense*, *Asplenium nemorale*, *Asplenium neolaserpitiifolium*, *Asplenium neomutijugum*, *Asplenium neovarians*, *Asplenium nesii*, *Asplenium nesioticum*, *Asplenium nidus* L., *Asplenium nigricans*,
20 *Asplenium niponicum*, *Asplenium normale*, *Asplenium normale* var. *angustum*, *Asplenium obesum*, *Asplenium oblongatum*, *Asplenium oblongifolium*, *Asplenium obovatum*, *Asplenium obscurum*, *Asplenium obscurum* var. *angustum*, *Asplenium obtusatum* var. *obtusatum*, *Asplenium obtusatum* var. *sphenoides*, *Asplenium obtusifolium* L., *Asplenium obtusissimum*, *Asplenium obversum*, *Asplenium ochraceum*, *Asplenium oellgaardii*, *Asplenium ofeliae*, *Asplenium oldhami*, *Asplenium oligosorum*, *Asplenium olivaceum*, *Asplenium onopteris* L., *Asplenium onustum*, *Asplenium ortegae*, *Asplenium otites*, *Asplenium palaciosii*, *Asplenium palmeri*, *Asplenium partitum*, *Asplenium parvisorum*, *Asplenium parviusculum*, *Asplenium parvulum*, *Asplenium patens*, *Asplenium paucifolium*, *Asplenium paucijugum*, *Asplenium paucivenosum*, *Asplenium pearcei*, *Asplenium pekinense*, *Asplenium pellucidum*, *Asplenium pendulum*, *Asplenium petiolulatum*, *Asplenium phyllitidis*, *Asplenium pimpinellifolium*, *Asplenium pinnatifidum*, *Asplenium pinnatum*, *Asplenium platyneuron*, *Asplenium platyneuron* var. *bacculum-rubrum*, *Asplenium platyneuron* var. *incisum*, *Asplenium platyphyllum*, *Asplenium plumbeum*, *Asplenium poloense*, *Asplenium polymeris*, *Asplenium polymorphum*, *Asplenium polyodon*, *Asplenium polyodon* var. *knudsenii*, *Asplenium polyodon* var. *nitidulum*, *Asplenium polyodon* var. *sectum*, *Asplenium polyodon* var. *subcaudatum*, *Asplenium polyphyllum*, *Asplenium poolii*, *Asplenium poolii* fo. *simplex*, *Asplenium poolii* var. *linearipinnatum*, *Asplenium potosinum*, *Asplenium potosinum* var. *incisum*, *Asplenium praegracile*, *Asplenium praemorsum*, *Asplenium preussii*, *Asplenium pringleanum*, *Asplenium pringlei*, *Asplenium prionitis*, *Asplenium procerum*, *Asplenium progrediens*, *Asplenium projectum*, *Asplenium prolongatum*, *Asplenium propinquum*, *Asplenium protensum*, *Asplenium pseudoangustum*, *Asplenium pseudoerectum*, *Asplenium pseudofontanum*, *Asplenium pseudolaserpitiifolium*, *Asplenium pseudonormale*, *Asplenium pseudopellucidum*, *Asplenium pseudopraemorsum*, *Asplenium pseudovarians*, *Asplenium pseudowilfordii*, *Asplenium pseudowrightii*, *Asplenium psilacrum*, *Asplenium pteropus*, *Asplenium pubirhizoma*, *Asplenium pulchellum*, *Asplenium pulchellum* var. *subhorizontale*, *Asplenium pulcherrimum*, *Asplenium pulicosum*, *Asplenium pulicosum* var. *maius*, *Asplenium pululahuae*, *Asplenium pumilum*, *Asplenium pumilum* var. *hymenophylloides*, *Asplenium pumilum* var. *laciniatum*, *Asplenium purdieanum*, *Asplenium purpurascens*, *Asplenium pyramidatum*, *Asplenium qiujiangense*, *Asplenium quercicola*, *Asplenium quitense*, *Asplenium raddianum*, *Asplenium radiatum*, *Asplenium radicans* L., *Asplenium radicans*, *Asplenium radicans* var. *costaricense*, *Asplenium radicans* var. *partitum*, *Asplenium radicans* var. *radicans*, *Asplenium radicans* var. *uniseriale*, *Asplenium recumbens*, *Asplenium reflexum*, *Asplenium regulare* var. *latior*, *Asplenium repandulum*, *Asplenium repens*, *Asplenium repente*, *Asplenium resiliens*, *Asplenium retusulum*, *Asplenium rhipidoneuron*, *Asplenium rhizophorum* L., *Asplenium rhizophyllum*, *Asplenium rhizophyllum* L., *Asplenium rhizophyllum* var. *proliferum*, *Asplenium rhomboideum*, *Asplenium rigidum*, *Asplenium riparium*, *Asplenium rivale*, *Asplenium rockii*, *Asplenium roemerianum*, *Asplenium roemerianum* var. *mindensis*, *Asplenium rosenstockianum*, *Asplenium rubinum*, *Asplenium ruizianum*, *Asplenium rusbyanum*, *Asplenium ruta-muraria* L., *Asplenium ruta-muraria* var. *cryptolepis*, *Asplenium rutaceum*, *Asplenium rutaceum* var. *disculiferum*, *Asplenium rutaefolium*, *Asplenium rutifolium*, *Asplenium salicifolium* L., *Asplenium salicifolium* var. *aequilaterale*, *Asplenium salicifolium* var. *salicifolium*, *Asplenium sampsoni*, *Asplenium sanchezii*, *Asplenium sanderi*, *Asplenium sandersonii*, *Asplenium sanguinolentum*, *Asplenium sarelii*, *Asplenium sarelii* var. *magnum*, *Asplenium sarelii* var. *sarelii*, *Asplenium saxicola*, *Asplenium scalifolium*, *Asplenium scandicinum*, *Asplenium schizophyllum*, *Asplenium schkuhrii*, *Asplenium sciadophilum*, *Asplenium scolopendrium* L., *Asplenium scortechinii*, *Asplenium seileri*, *Asplenium semipinnatum*, *Asplenium septentrionale*, *Asplenium serra*, *Asplenium serra* var. *imrayanum*, *Asplenium serratissimum*, *Asplenium serratum* L., *Asplenium serratum* var. *caudatum*, *Asplenium serricula*, *Asplenium sessilifolium*, *Asplenium sessilifolium* var. *guatemalense*, *Asplenium sessilifolium* var. *minus*, *Asplenium sessilifolium* var. *occidentale*, *Asplenium sessilipinnum*, *Asplenium setosum*, *Asplenium*

shepherdii, *Asplenium shepherdii* var. *bipinnatum*, *Asplenium shepherdii* var. *flagelliferum*, *Asplenium shikokianum*,
Asplenium simii, *Asplenium simonsianum*, *Asplenium sintenisii*, *Asplenium skinneri*, *Asplenium skinneri*, *Asplenium*
sodiroi, *Asplenium soleirolloides*, *Asplenium solidum* var. *stenophyllum*, *Asplenium solmsii*, *Asplenium* sp.-N.-Halle-
 5 *2234*, *Asplenium spathulinum*, *Asplenium spectabile*, *Asplenium speluncae*, *Asplenium sphaerosporum*, *Asplenium*
sphenotomum, *Asplenium spinescens*, *Asplenium splendens*, *Asplenium sprucei*, *Asplenium squamosum* L., *Asplenium*
standleyi, *Asplenium stellatum*, *Asplenium stenocarpum*, *Asplenium stoloniferum*, *Asplenium stolonipes*, *Asplenium*
 10 *striatum* L., *Asplenium stuebelianum*, *Asplenium stuhlmannii*, *Asplenium suave*, *Asplenium subalatum*, *Asplenium sub-*
crenatum, *Asplenium subdigitatum*, *Asplenium subdimidiatum*, *Asplenium subintegrum*, *Asplenium sublaserpitiifolium*,
Asplenium sublongum, *Asplenium subnudum*, *Asplenium suborbiculare*, *Asplenium subtenuifolium*, *Asplenium subtile*,
 15 *Asplenium subtoramanum*, *Asplenium subtrapezoideum*, *Asplenium subvarians*, *Asplenium sulcatum*, *Asplenium syl-*
vaticum, *Asplenium szechuanense*, *Asplenium taiwanense*, *Asplenium tenerrimum*, *Asplenium tenerum*, *Asplenium*
tenuicaule, *Asplenium tenuifolium*, *Asplenium tenuifolium* var. *minor*, *Asplenium tenuifolium* var. *tenuifolium*, *Asplenium*
tenuissimum, *Asplenium ternatum*, *Asplenium theciferum*, *Asplenium theciferum* var. *concinnum*, *Asplenium thunbergii*,
 20 *Asplenium tianmushanense*, *Asplenium tianshanense*, *Asplenium tibeticum*, *Asplenium tocoraniense*, *Asplenium toram-*
anum, *Asplenium trapezoideum*, *Asplenium tricholepis*, *Asplenium trichomanes* L., *Asplenium trichomanes* subsp. *in-*
expectans, *Asplenium trichomanes* subsp. *quadrivalens*, *Asplenium trichomanes* subsp. *trichomanes*, *Asplenium tri-*
chomanes var. *harovii*, *Asplenium trichomanes* var. *herbaceum*, *Asplenium trichomanes* var. *repens*, *Asplenium tri-*
 25 *chomanes* var. *viridissimum*, *Asplenium trichomanes-dentatum* L., *Asplenium trigonopterum*, *Asplenium trilobatum*, *As-*
plenium trilobum, *Asplenium triphyllum*, *Asplenium triphyllum* var. *compactum*, *Asplenium triphyllum* var. *gracillimum*,
 30 *Asplenium triphyllum* var. *herbaceum*, *Asplenium tripteropus*, *Asplenium triquetrum*, *Asplenium truncorum*, *Asplenium*
tsaratananense, *Asplenium tucumanense*, *Asplenium tuerckheimii*, *Asplenium tunquiniense*, *Asplenium ulbrichtii*, *As-*
plenium ultimum, *Asplenium unilaterale*, *Asplenium unilaterale* var. *decurrens*, *Asplenium unilaterale* var. *udum*, *Asple-*
 35 *nium unilaterale* var. *unilaterale*, *Asplenium uniseriale*, *Asplenium uropterum*, *Asplenium vagans*, *Asplenium vareschi-*
anum, *Asplenium variabile* var. *paucijugum*, *Asplenium variabile* var. *variabile*, *Asplenium varians* subsp. *fimbriatum*,
 40 *Asplenium varians*, *Asplenium vastum*, *Asplenium venturae*, *Asplenium venulosum*, *Asplenium verapax*, *Asplenium*
vesiculosum, *Asplenium vespertinum*, *Asplenium villosum*, *Asplenium virens*, *Asplenium viride*, *Asplenium viridifrons*,
Asplenium virillae, *Asplenium viviparioides*, *Asplenium viviparum*, *Asplenium viviparum* var. *viviparum*, *Asplenium vivi-*
 45 *parum* var. *lineatu*, *Asplenium volubile*, *Asplenium vulcanicum*, *Asplenium wacketii*, *Asplenium wagneri*, *Asplenium*
wallichianum, *Asplenium warneckeii*, *Asplenium wilfordii*, *Asplenium williamsii*, *Asplenium wrightii*, *Asplenium wrightio-*
 50 *ides*, *Asplenium wuliangshanense*, *Asplenium xianqianense*, *Asplenium xinjiangense*, *Asplenium xinyiense*, *Asplenium*
yelagagense, *Asplenium yoshinagae*, *Asplenium yunnanense*, *Asplenium zamiifolium*, *Asplenium zanzibaricum*, *Asple-*
nium biscayneanum, *Asplenium curtissii*, *Asplenium ebenoides*, *Asplenium herb-wagneri*, *Asplenium heteroresiliens*,
Asplenium kenzoi, *Asplenium plenum*, *Asplenium wangii*, and *Asplenium* × *clermontiae*, *Asplenium* × *gravesii*.

[0066] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in
 35 the Order Polypodiales, Family *Blechnaceae*, Genus *Blechnum* L. In some aspects the nucleic acid molecule encoding
 a PtlP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Blechnaceae*, Genus *Blechnum*
 L. selected from but not limited to *Blechnum amabile*, *Blechnum appendiculatum*, *Blechnum articulatum*, *Blechnum*
australe, *Blechnum austrobrasiliense*, *Blechnum binervatum*, *Blechnum blechnoides*, *Blechnum brasiliense*, *Blechnum*
 40 *capense*, *Blechnum cartilagineum*, *Blechnum castaneum*, *Blechnum chambersii*, *Blechnum chilense*, *Blechnum colen-*
soi, *Blechnum contiguum*, *Blechnum cordatum*, *Blechnum coriaceum*, *Blechnum discolor*, *Blechnum doodioides*, *Blech-*
num durum, *Blechnum eburneum*, *Blechnum ensiforme*, *Blechnum filiforme*, *Blechnum fluviatile*, *Blechnum fragile*, *Blech-*
num fraseri, *Blechnum fullagari*, *Blechnum gibbum*, *Blechnum glandulosum*, *Blechnum gracile*, *Blechnum hancockii*,
 45 *Blechnum hastatum*, *Blechnum howeanum*, *Blechnum indicum*, *Blechnum kunthianum*, *Blechnum laevigatum*, *Blechnum*
loxense, *Blechnum magellanicum*, *Blechnum membranaceum*, *Blechnum microbasis*, *Blechnum microphyllum*, *Blech-*
num milnei, *Blechnum minus*, *Blechnum mochaenum*, *Blechnum montanum*, *Blechnum moorei*, *Blechnum moritzianum*,
Blechnum nigrum, *Blechnum niponicum*, *Blechnum norfolkianum*, *Blechnum novae-zelandiae*, *Blechnum nudum*, *Blech-*
 50 *num obtusatum*, *Blechnum occidentale*, *Blechnum oceanicum*, *Blechnum orientale*, *Blechnum patersonii*, *Blechnum*
penna-marina, *Blechnum polypodioides*, *Blechnum procerum*, *Blechnum punctulatum*, *Blechnum sampaioanum*, *Blech-*
num schiedeanum, *Blechnum schomburgkii*, *Blechnum serrulatum*, *Blechnum simillimum*, *Blechnum spicant*, *Blechnum*
stipitellatum, *Blechnum tabulare*, *Blechnum triangularifolium*, *Blechnum vieillardii*, *Blechnum vulcanicum*, *Blechnum*
wattsii, *Blechnum whelanii*, and *Blechnum wurunuran*.

[0067] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in
 the Order Polypodiales, Family *Dryopteridaceae* Genus *Acrophorus*, Genus *Acrorumohra*, Genus *Anapausia*, Genus
 55 *Arachniodes*, Genus *Bolbitis*, Genus *Ctenitis*, Genus *Cyclodium*, Genus *Cyrtogonellum*, Genus *Cyrtomidictyum*, Genus
Cyrtomium, Genus *Diacalpe*, Genus *Didymochlaena*, Genus *Dryopsis*, Genus *Dryopteris*, Genus *Elaphoglossum*, Genus
Hypodematium, Genus *Lastreopsis*, Genus *Leptorumohra*, Genus *Leucostegia*, Genus *Lithostegia*, Genus *Lomagram-*
ma, Genus *Maxonia*, Genus *Megalastrum*, Genus *Olfersia*, Genus *Peranema*, Genus *Phanerophlebia*, Genus *Phaner-*
ophlebiopsis, Genus *Polybotrya*, Genus *Polystichopsis*, Genus *Polystichum*, Genus *Rumohra*, Genus *Sorolepidium*,

Genus *Stigmatopteris* or Genus *Teratophyllum*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Dryopteridaceae*, Genus *Bolbitis*, selected from but not limited to *Bolbitis acrostichoides*, *Bolbitis aliena*, *Bolbitis angustipinna*, *Bolbitis appendiculata*, *Bolbitis auriculata*, *Bolbitis bernoullii*, *Bolbitis bipinnatifida*, *Bolbitis cadieri*, *Bolbitis christensenii*, *Bolbitis confertifolia*, *Bolbitis costata*, *Bolbitis crispatula*, *Bolbitis fluviatilis*, *Bolbitis gaboensis*, *Bolbitis gemmifera*, *Bolbitis hainanensis*, *Bolbitis hastata*, *Bolbitis hekouensis*, *Bolbitis hemiotis*, *Bolbitis heteroclita*, *Bolbitis heudelotii*, *Bolbitis humblotii*, *Bolbitis interlineata*, *Bolbitis latipinna*, *Bolbitis laxireticulata*, *Bolbitis lindigii*, *Bolbitis lonchophora*, *Bolbitis longiflagellata*, *Bolbitis major*, *Bolbitis media*, *Bolbitis nicotianifolia*, *Bolbitis nodiflora*, *Bolbitis novoguineensis*, *Bolbitis oligarchica*, *Bolbitis palustris*, *Bolbitis pandurifolia*, *Bolbitis pergamentacea*, *Bolbitis portoricensis*, *Bolbitis presliana*, *Bolbitis quoyana*, *Bolbitis rawsonii*, *Bolbitis repanda*, *Bolbitis rhizophylla*, *Bolbitis riparia*, *Bolbitis rivularis*, *Bolbitis sagenioides*, *Bolbitis salicina*, *Bolbitis scalpturata*, *Bolbitis scandens*, *Bolbitis semicordata*, *Bolbitis semipinnatifida*, *Bolbitis serrata*, *Bolbitis serratifolia*, *Bolbitis simplex*, *Bolbitis sinensis*, *Bolbitis singaporensis*, *Bolbitis sinuata*, *Bolbitis subcordata*, *Bolbitis subcrenata*, *Bolbitis taylorii*, *Bolbitis tibetica*, *Bolbitis tonkinensis*, *Bolbitis umbrosa*, *Bolbitis vanuaensis*, and *Bolbitis virens*.

[0068] In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Lomariopsidaceae*, Genus *Nephrolepis*. In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Lomariopsidaceae*, Genus *Nephrolepis* is selected from but not limited to *Nephrolepis abrupta*, *Nephrolepis acuminata*, *Nephrolepis acutifolia*, *Nephrolepis arida*, *Nephrolepis arthropteroides*, *Nephrolepis biserrata* var. *auriculata*, *Nephrolepis brownii*, *Nephrolepis celebica*, *Nephrolepis clementis*, *Nephrolepis cordifolia*, *Nephrolepis davalliae*, *Nephrolepis davallioides*, *Nephrolepis dayakorum*, *Nephrolepis delicatula*, *Nephrolepis dicksonioides*, *Nephrolepis duffii*, *Nephrolepis exaltata* ssp. *exaltata* ssp. *Hawaiiensis*, *Nephrolepis falcata*, *Nephrolepis falciformis*, *Nephrolepis glabra*, *Nephrolepis hirsutula*, *Nephrolepis humatoides*, *Nephrolepis iridescens*, *Nephrolepis kurotawae*, *Nephrolepis laurifolia*, *Nephrolepis lauterbachii*, *Nephrolepis lindsayae*, *Nephrolepis multifida*, *Nephrolepis multiflora*, *Nephrolepis niphoboloides*, *Nephrolepis obliterate*, *Nephrolepis paludosa*, *Nephrolepis pectinata*, *Nephrolepis pendula*, *Nephrolepis persicifolia*, *Nephrolepis pickelii*, *Nephrolepis pilosula*, *Nephrolepis pubescens*, *Nephrolepis pumicicola*, *Nephrolepis radicans*, *Nephrolepis rivularis*, *Nephrolepis rosenstockii*, *Nephrolepis saligna*, *Nephrolepis schlechteri*, *Nephrolepis serrate*, *Nephrolepis thomsoni*, *Nephrolepis undulata* var. *aureoglandulosa*, *Nephrolepis x averyi*, *Nephrolepis x copelandii*, and *Nephrolepis x medlerae*.

[0069] In some aspects the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae* Genus *Campyloneurum*, Genus *Drynaria*, Genus *Lepisorus*, Genus *Microgramma*, Genus *Microsorium*, Genus *Neurodium*, Genus *Niphidium*, Genus *Pecluma* M.G., Genus *Phlebodium*, Genus *Phymatosorus*, Genus *Platynerium*, Genus *Pleopeltis*, Genus *Polypodium* L.

[0070] In some embodiments the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae*, Genus *Polypodium* L. In some embodiments the nucleic acid molecule encoding a PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae*, Genus *Polypodium* L. selected from but not limited to *Polypodium absidatum*, *Polypodium acutifolium*, *Polypodium adiantiforme*, *Polypodium aequale*, *Polypodium affine*, *Polypodium albidopaleatum*, *Polypodium alcicorne*, *Polypodium alfarii*, *Polypodium alfredii*, *Polypodium alfredii* var. *curtii*, *Polypodium allosuroides*, *Polypodium alsophilicola*, *Polypodium amami-anum*, *Polypodium amoenum*, *Polypodium amorphum*, *Polypodium anetioides*, *Polypodium anfractuosum*, *Polypodium anguinum*, *Polypodium angustifolium* f. *remotifolia*, *Polypodium angustifolium* var. *amphostenon*, *Polypodium angustifolium* var. *heterolepis*, *Polypodium angustifolium* var. *monstrosa*, *Polypodium angustipaleatum*, *Polypodium angustissimum*, *Polypodium anisomeron* var. *pectinatum*, *Polypodium antioquianum*, *Polypodium aoristorum*, *Polypodium apagolepis*, *Polypodium apicidens*, *Polypodium apiculatum*, *Polypodium apoense*, *Polypodium appalachianum*, *Polypodium appressum*, *Polypodium arenarium*, *Polypodium argentinum*, *Polypodium argutum*, *Polypodium armatum*, *Polypodium aromaticum*, *Polypodium aspersum*, *Polypodium assurgens*, *Polypodium atrum*, *Polypodium auriculatum*, *Polypodium balaonense*, *Polypodium balliviani*, *Polypodium bamleri*, *Polypodium bangii*, *Polypodium bartlettii*, *Polypodium basale*, *Polypodium bernoullii*, *Polypodium biauratum*, *Polypodium bifrons*, *Polypodium blepharodes*, *Polypodium bolivari*, *Polypodium bolivianum*, *Polypodium bolobense*, *Polypodium bombycinum*, *Polypodium bombycinum* var. *insularum*, *Polypodium bradeorum*, *Polypodium bryophilum*, *Polypodium bryopodium*, *Polypodium buchtienii*, *Polypodium buesii*, *Polypodium bulbotrichum*, *Polypodium caceresii*, *Polypodium californicum* f. *brauscombii*, *Polypodium californicum* f. *parsonsiaae*, *Polypodium californicum*, *Polypodium calophlebium*, *Polypodium calvum*, *Polypodium camptophyllum* var. *abbreviatum*, *Polypodium capitellatum*, *Polypodium carpinterae*, *Polypodium chachapoyense*, *Polypodium chartaceum*, *Polypodium chimantense*, *Polypodium chiricanum*, *Polypodium choquetangense*, *Polypodium christensenii*, *Polypodium christii*, *Polypodium chrysotrichum*, *Polypodium ciliolepis*, *Polypodium cinerascens*, *Polypodium collinsii*, *Polypodium colysoides*, *Polypodium confluens*, *Polypodium conforme*, *Polypodium confusum*, *Polypodium congregatifolium*, *Polypodium connellii*, *Polypodium consimile* var. *bourgaeum*, *Polypodium consimile* var. *minor*, *Polypodium conterminans*, *Polypodium contiguum*, *Polypodium cookii*, *Polypodium coriaceum*, *Polypodium coronans*, *Polypodium costaricense*, *Polypodium costatum*, *Polypodium crassifolium* f. *angustissimum*, *Polypodium crassifolium* var. *longipes*, *Polypodium crassulum*, *Polypodium craterisorum*, *Polypodium cryptum*, *Polypodium crystalloneuron*, *Polypodium cucullatum*

var. *planum*, *Polypodium cuencanum*, *Polypodium cumingianum*, *Polypodium cupreolepis*, *Polypodium curranii*, *Polypodium curvans*, *Polypodium cyathicola*, *Polypodium cyathisorum*, *Polypodium cyclocolpon*, *Polypodium daguense*, *Polypodium damunense*, *Polypodium dareiformioides*, *Polypodium dasypleura*, *Polypodium decipiens*, *Polypodium decorum*, *Polypodium delicatulum*, *Polypodium deltoideum*, *Polypodium demeraranum*, *Polypodium denticulatum*, *Polypodium diaphanum*, *Polypodium dilatatum*, *Polypodium dispersum*, *Polypodium dissectum*, *Polypodium dissimulans*, *Polypodium dolichosorum*, *Polypodium doloreense*, *Polypodium donnell-smithii*, *Polypodium drymoglossoides*, *Polypodium ebeninum*, *Polypodium eggersii*, *Polypodium elmeri*, *Polypodium elongatum*, *Polypodium enterosoroides*, *Polypodium erubescens*, *Polypodium erythrolepis*, *Polypodium erythrotrichum*, *Polypodium eurybasis*, *Polypodium eurybasis* var. *villosum*, *Polypodium exornans*, *Polypodium falcoideum*, *Polypodium fallacissimum*, *Polypodium farinosum*, *Polypodium faucium*, *Polypodium feei*, *Polypodium ferrugineum*, *Polypodium feuillei*, *Polypodium firmulum*, *Polypodium firmum*, *Polypodium flaccidum*, *Polypodium flagellare*, *Polypodium flexuosum*, *Polypodium flexuosum* var. *ekmanii*, *Polypodium forbesii*, *Polypodium formosanum*, *Polypodium fraxinifolium* subsp. *articulatum*, *Polypodium fraxinifolium* subsp. *luridum*, *Polypodium fructuosum*, *Polypodium fucoides*, *Polypodium fulvescens*, *Polypodium galeottii*, *Polypodium glaucum*, *Polypodium glycyrrhiza*, *Polypodium gracillimum*, *Polypodium gramineum*, *Polypodium grandifolium*, *Polypodium gratum*, *Polypodium graveolens*, *Polypodium griseo-nigrum*, *Polypodium griseum*, *Polypodium guttatum*, *Polypodium haallioanum*, *Polypodium hammatisorum*, *Polypodium hancockii*, *Polypodium haplophlebicum*, *Polypodium harrisii*, *Polypodium hastatum* var. *simplex*, *Polypodium hawaiiense*, *Polypodium heanophyllum*, *Polypodium helleri*, *Polypodium hemionitidium*, *Polypodium henryi*, *Polypodium herzogii*, *Polypodium hesperium*, *Polypodium hessii*, *Polypodium hombersleyi*, *Polypodium hostmannii*, *Polypodium humile*, *Polypodium hyalinum*, *Polypodium iboense*, *Polypodium induens* var. *subdentatum*, *Polypodium insidiosum*, *Polypodium insigne*, *Polypodium intermedium* subsp. *masafueranum* var. *obtusesserratum*, *Polypodium intramarginale*, *Polypodium involutum*, *Polypodium itatiayense*, *Polypodium javanicum*, *Polypodium juglandifolium*, *Polypodium kaniense*, *Polypodium knowltoniorum*, *Polypodium kyimbilense*, *Polypodium l'herminieri* var. *costaricense*, *Polypodium lachniferum* f. *incurvata*, *Polypodium lachniferum* var. *glabrescens*, *Polypodium lachnopus*, *Polypodium lanceolatum* var. *complanatum*, *Polypodium lanceolatum* var. *trichophorum*, *Polypodium latevagans*, *Polypodium laxifrons*, *Polypodium laxifrons* var. *lividum*, *Polypodium lehmannianum*, *Polypodium leiorhizum*, *Polypodium leptopodon*, *Polypodium leuconeuron* var. *angustifolia*, *Polypodium leuconeuron* var. *latifolium*, *Polypodium leucosticta*, *Polypodium limulum*, *Polypodium lindigii*, *Polypodium lineatum*, *Polypodium lomarioides*, *Polypodium longifrons*, *Polypodium loretense*, *Polypodium loriceum* var. *umbraticum*, *Polypodium loriforme*, *Polypodium loxogramme* f. *gigas*, *Polypodium ludens*, *Polypodium luzonicum*, *Polypodium lycopodioides* f. *obtusum*, *Polypodium lycopodioides* L., *Polypodium macrolepis*, *Polypodium macrophyllum*, *Polypodium macrosorum*, *Polypodium macrosphaerum*, *Polypodium maculosum*, *Polypodium madrense*, *Polypodium manmeiense*, *Polypodium margaritifera*, *Polypodium maritimum*, *Polypodium martensii*, *Polypodium majoris*, *Polypodium megalolepis*, *Polypodium melanotrichum*, *Polypodium menisciifolium* var. *pubescens*, *Polypodium meniscioides*, *Polypodium merrillii*, *Polypodium mettenii*, *Polypodium mexiae*, *Polypodium microsorum*, *Polypodium militare*, *Polypodium minimum*, *Polypodium minusculum*, *Polypodium mixtum*, *Polypodium mollendense*, *Polypodium mollissimum*, *Polypodium moniliforme* var. *minus*, *Polypodium monooides*, *Polypodium monticola*, *Polypodium montigenum*, *Polypodium moritzianum*, *Polypodium moultonii*, *Polypodium multicaudatum*, *Polypodium multilineatum*, *Polypodium multisorum*, *Polypodium munchii*, *Polypodium muscoides*, *Polypodium myriolepis*, *Polypodium myriophyllum*, *Polypodium myriotrichum*, *Polypodium nematorhizon*, *Polypodium nemorale*, *Polypodium nesioticum*, *Polypodium nigrescentium*, *Polypodium nigripes*, *Polypodium nigrocinctum*, *Polypodium nibatam*, *Polypodium nitidissimum*, *Polypodium nitidissimum* var. *latior*, *Polypodium nubrigenum*, *Polypodium oligolepis*, *Polypodium oligosorum*, *Polypodium oligosorum*, *Polypodium olivaceum*, *Polypodium olivaceum* var. *elatum*, *Polypodium oodes*, *Polypodium oosphaerum*, *Polypodium oreophilum*, *Polypodium ornatissimum*, *Polypodium ornatum*, *Polypodium ovatum*, *Polypodium oxylobum*, *Polypodium oxypholis*, *Polypodium pakkaense*, *Polypodium pallidum*, *Polypodium palmatopedatum*, *Polypodium palmeri*, *Polypodium panamense*, *Polypodium parvum*, *Polypodium patagonicum*, *Polypodium paucisorum*, *Polypodium pavonianum*, *Polypodium pectinatum* var. *caliense*, *Polypodium pectinatum* var. *hispidum*, *Polypodium pellucidum*, *Polypodium pendulum* var. *boliviense*, *Polypodium percrassum*, *Polypodium perpusillum*, *Polypodium peruvianum* var. *subgibbosum*, *Polypodium phyllitidis* var. *elongatum*, *Polypodium pichinchense*, *Polypodium pilosissimum*, *Polypodium pilosissimum* var. *glabriusculum*, *Polypodium pilosissimum* var. *tunguraquensis*, *Polypodium pityrolepis*, *Polypodium platyphyllum*, *Polypodium playfairii*, *Polypodium plebeium* var. *cooperi*, *Polypodium plectolepidioides*, *Polypodium pleolepis*, *Polypodium plesiosorum* var. *i*, *Polypodium podobasis*, *Polypodium podocarpum*, *Polypodium poloense*, *Polypodium polydatylon*, *Polypodium polypodioides* var. *aciculare*, *Polypodium polypodioides* var. *michauxianum*, *Polypodium praetermissum*, *Polypodium preslianum* var. *immersum*, *Polypodium procerum*, *Polypodium procerum*, *Polypodium productum*, *Polypodium productum*, *Polypodium prolongilobum*, *Polypodium propinguum*, *Polypodium proteus*, *Polypodium pruinatum*, *Polypodium pseudocapillare*, *Polypodium pseudofraternum*, *Polypodium pseudonutans*, *Polypodium pseudoserratum*, *Polypodium pulcherrimum*, *Polypodium pulogense*, *Polypodium pungens*, *Polypodium purpusii*, *Polypodium radicale*, *Polypodium randallii*, *Polypodium ratiborii*, *Polypodium reclinatum*, *Polypodium recreense*, *Polypodium repens* var. *abruptum*, *Polypodium revolvens*, *Polypodium rhachipterygium*, *Polypodium rhomboideum*, *Polypodium rigens*, *Polypodium robustum*, *Polypodium roraimense*, *Polypodium roraimense*, *Polypodium ro-*

sei, *Polypodium rosenstockii*, *Polypodium rubidum*, *Polypodium rudimentum*, *Polypodium rusbyi*, *Polypodium sablanianum*, *Polypodium sarmentosum*, *Polypodium saxicola*, *Polypodium schenckii*, *Polypodium schlechteri*, *Polypodium scolopendria*, *Polypodium scolopendria*, *Polypodium scolopendrium*, *Polypodium scouleri*, *Polypodium scutulatum*, *Polypodium segregatum*, *Polypodium semihirsutum*, *Polypodium semihirsutum* var. *fuscetosum*, *Polypodium senile* var. *minor*, *Polypodium sericeolanatum*, *Polypodium serraeforme*, *Polypodium serricula*, *Polypodium sesquipedala*, *Polypodium sessilifolium*, *Polypodium setosum* var. *calvum*, *Polypodium setulosum*, *Polypodium shaferi*, *Polypodium sibomense*, *Polypodium siccum*, *Polypodium simacense*, *Polypodium simulans*, *Polypodium singeri*, *Polypodium sinicum*, *Polypodium sintenisii*, *Polypodium skutchii*, *Polypodium sloanei*, *Polypodium sodiroi*, *Polypodium sordidulum*, *Polypodium sordidum*, *Polypodium sphaeropteroides*, *Polypodium sphenodes*, *Polypodium sprucei*, *Polypodium sprucei* var. *furcativenosa*, *Polypodium steirolepis*, *Polypodium stenobasis*, *Polypodium stenolepis*, *Polypodium stenopterum*, *Polypodium subcapillare*, *Polypodium subflabelliforme*, *Polypodium subhemionitidium*, *Polypodium subinaequale*, *Polypodium subintegrum*, *Polypodium subspathulatum*, *Polypodium subtile*, *Polypodium subvestitum*, *Polypodium subviride*, *Polypodium superficiale* var. *attenuatum*, *Polypodium superficiale* var. *chinensis*, *Polypodium sursumcurrens*, *Polypodium tablazianum*, *Polypodium taenifolium*, *Polypodium tamandarei*, *Polypodium tatei*, *Polypodium tenuiculum* var. *acrosora*, *Polypodium tenuiculum* var. *brasiliense*, *Polypodium tenuilore*, *Polypodium tenuinerve*, *Polypodium tepuiense*, *Polypodium teresae*, *Polypodium tetragonum* var. *incompletum*, *Polypodium thysanolepis* var. *bipinnatifidum*, *Polypodium thysanolepis*, var. *thysanolepis*, *Polypodium thysanolepsi*, *Polypodium tobagense*, *Polypodium trichophyllum*, *Polypodium tridactylum*, *Polypodium tridentatum*, *Polypodium trifurcatum* var. *brevipes*, *Polypodium triglossum*, *Polypodium truncatum*, *Polypodium truncicola* var. *major*, *Polypodium truncicola* var. *minor*, *Polypodium tuberosum*, *Polypodium tunguraguae*, *Polypodium turquinum*, *Polypodium turrialbae*, *Polypodium ursipes*, *Polypodium vagans*, *Polypodium valdealatum*, *Polypodium versteegii*, *Polypodium villagrani*, *Polypodium virginianum* f. *cambroideum*, *Polypodium virginianum* f. *periferens*, *Polypodium vittarioides*, *Polypodium vulgare*, *Polypodium vulgare* L., *Polypodium vulgare* subsp. *oreophilum*, *Polypodium vulgare* var. *acuminatum*, *Polypodium vulpinum*, *Polypodium williamsii*, *Polypodium wobbenense*, *Polypodium x fallacissimum-guttatum*, *Polypodium xantholepis*, *Polypodium xiphopteris*, *Polypodium yaru-malense*, *Polypodium yungense*, and *Polypodium zosteriforme*.

[0071] In some aspects the PtlP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platyserium*. In some aspects the PtlP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platyserium* selected from but not limited to *Platyserium alcicorne*, *Platyserium andinum*, *Platyserium angolense*, *Platyserium bifurcatum*, *Platyserium coronarium*, *Platyserium elephantotis*, *Platyserium ellisii*, *Platyserium grande*, *Platyserium hillii*, *Platyserium holttumii*, *Platyserium madagascariense*, *Platyserium quadridichotomum*, *Platyserium ridleyi*, *Platyserium* sp. ES-2011, *Platyserium stemaria*, *Platyserium superbum*, *Platyserium veitchii*, *Platyserium wallichii*, *Platyserium wandae*, *Platyserium wilhelminae-reginae*, and *Platyserium willinckii*.

[0072] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Division Lycophyta.

[0073] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Class Isoetopsida or Class Lycopodiopsida.

[0074] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Class Isoetopsida Order Selaginales. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Class Isoetopsida, Order Selaginales, Family Selaginellaceae. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Genus *Selaginella*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a *Selaginella* species selected from but not limited to *Selaginella acanthonota*, *Selaginella apoda*, *Selaginella arbuscula*, *Selaginella arenicola*, *Selaginella arizonica*, *Selaginella armata*, *Selaginella asprella*, *Selaginella bififormis*, *Selaginella bigelovii*, *Selaginella braunii*, *Selaginella cinerascens*, *Selaginella cordifolia*, *Selaginella deflexa*, *Selaginella delicatula*, *Selaginella densa*, *Selaginella douglasii*, *Selaginella eatonii*, *Selaginella eclipses*, *Selaginella eremophila*, *Selaginella erythropus*, *Selaginella flabellata*, *Selaginella hansenii*, *Selaginella heterodonta*, *Selaginella kraussiana*, *Selaginella krugii*, *Selaginella laxifolia*, *Selaginella lepidophylla*, *Selaginella leucobryoides*, *Selaginella ludoviciana*, *Selaginella mutica*, *Selaginella oregana*, *Selaginella ovifolia*, *Selaginella pallescens*, *Selaginella peruviana*, *Selaginella pilifera*, *Selaginella plana*, *Selaginella plumosa*, *Selaginella pulcherrima*, *Selaginella rupestris*, *Selaginella rupincola*, *Selaginella scopulorum*, *Selaginella selaginoides*, *Selaginella sibirica*, *Selaginella standleyi*, *Selaginella stellata*, *Selaginella subcaulescens*, *Selaginella substipitata*, *Selaginella tenella*, *Selaginella tortipila*, *Selaginella uliginosa*, *Selaginella umbrosa*, *Selaginella uncinata*, *Selaginella underwoodii*, *Selaginella utahensis*, *Selaginella victoriae*, *Selaginella viridissima*, *Selaginella wallacei*, *Selaginella watsonii*, *Selaginella weatherbiana*, *Selaginella willdenowii*, *Selaginella wrightii* and *Selaginella X neomexicana*.

[0075] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Class Lycopodiopsida, Order Lycopodiales. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a fern species in the Class Lycopodiopsida, Order Lycopodiales Family Lycopodiaceae or Family Huperziaceae. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the

Genus *Austrolycopodium*, *Dendrolycopodium*, *Diphasiastrum*, *Diphasium*, *Huperzia*, *Lateristachys*, *Lycopodiastrum*, *Lycopodiella*, *Lycopodium*, *Palhinhaea*, *Pseudodiphasium*, *Pseudolycopodiella*, *Pseudolycopodium* or *Spinulum*.

[0076] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Genus *Lycopodium*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a *Lycopodium* species selected from but not limited to *Lycopodium alpinum* L., *Lycopodium annotinum* L., *Lycopodium clavatum* L., *Lycopodium complanatum* L., *Lycopodium dendroideum* Michx., *Lycopodium digitatum*, *Lycopodium xhabereri*, *Lycopodium hickeyi*, *Lycopodium xissleri*, *Lycopodium lagopus*, *Lycopodium obscurum* L., *Lycopodium phlegmaria* L., *Lycopodium sabinifolium*, *Lycopodium sitchense*, *Lycopodium tristachyum*, *Lycopodium venustulum*, *Lycopodium venustulum* var. *venustulum*, *Lycopodium venustulum* var. *verticale*, *Lycopodium volubile* and *Lycopodium* × *zeilleri*.

[0077] In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species in the Genus *Huperzia*. In some aspects the nucleic acid molecule encoding a PtlP-65 polypeptide is derived from a species selected from but not limited to *Huperzia appressa*, *Huperzia arctica*, *Huperzia attenuata*, *Huperzia australiana*, *Huperzia balansae*, *Huperzia billardierei*, *Huperzia brassii*, *Huperzia campiana*, *Huperzia capellae*, *Huperzia carinata*, *Huperzia cf. carinata* ARF000603, *Huperzia cf. nummulariifolia* ARF001140, *Huperzia cf. phlegmaria* ARF000717, *Huperzia cf. phlegmaria* ARF000771, *Huperzia cf. phlegmaria* ARF000785, *Huperzia cf. phlegmaria* ARF001007, *Huperzia cf. phlegmaria* ARF002568, *Huperzia cf. phlegmaria* ARF002703, *Huperzia cf. phlegmaria* Wikstrom 1998, *Huperzia chinensis*, *Huperzia compacta*, *Huperzia crassa*, *Huperzia crispata*, *Huperzia cryptomeriana*, *Huperzia cumingii*, *Huperzia dacrydioides*, *Huperzia dalhousieana*, *Huperzia dichotoma*, *Huperzia emeiensis*, *Huperzia ericifolia*, *Huperzia eversa*, *Huperzia fargesii*, *Huperzia fordii*, *Huperzia funiformis*, *Huperzia goebellii*, *Huperzia haleakalae*, *Huperzia hamiltonii*, *Huperzia heteroclita*, *Huperzia hippuridea*, *Huperzia hippuris*, *Huperzia holstii*, *Huperzia horizontalis*, *Huperzia hunanensis*, *Huperzia hystrix*, *Huperzia lindenii*, *Huperzia linifolia*, *Huperzia lockyeri*, *Huperzia lucidula*, *Huperzia mingcheensis*, *Huperzia miyoshiana*, *Huperzia nanchuanensis*, *Huperzia nummulariifolia*, *Huperzia obtusifolia*, *Huperzia ophioglossoides*, *Huperzia petiolata*, *Huperzia phlegmaria*, *Huperzia phlegmarioides*, *Huperzia phyllantha*, *Huperzia pinifolia*, *Huperzia polydactyla*, *Huperzia prolifera*, *Huperzia reflexa*, *Huperzia rosenstockiana*, *Huperzia rufescens*, *Huperzia salvinoides*, *Huperzia sarmentosa*, *Huperzia selago*, *Huperzia serrata*, *Huperzia sieboldii*, *Huperzia somae*, *Huperzia squarrosa*, *Huperzia subulata*, *Huperzia sutchueniana*, *Huperzia tauri*, *Huperzia taxifolia*, *Huperzia tenuis*, *Huperzia tetragona*, *Huperzia tetrasticha*, *Huperzia unguiculata*, *Huperzia varia*, *Huperzia verticillata* and *Huperzia wilsonii*.

[0078] Also provided are nucleic acid molecules that encode transcription and/or translation products that are subsequently spliced to ultimately produce functional PtlP-50 polypeptides or PtlP-65 polypeptides. Splicing can be accomplished in vitro or in vivo, and can involve cis- or trans-splicing. The substrate for splicing can be polynucleotides (e.g., RNA transcripts) or polypeptides. An example of cis-splicing of a polynucleotide is where an intron inserted into a coding sequence is removed and the two flanking exon regions are spliced to generate a PtlP-50 polypeptide or PtlP-65 polypeptide encoding sequence. An example of trans splicing would be where a polynucleotide is encrypted by separating the coding sequence into two or more fragments that can be separately transcribed and then spliced to form the full-length pesticidal encoding sequence. The use of a splicing enhancer sequence, which can be introduced into a construct, can facilitate splicing either in cis or trans-splicing of polypeptides (US Patent Numbers 6,365,377 and 6,531,316). Thus, in some embodiments the polynucleotides do not directly encode a full-length PtlP-50 polypeptide or PtlP-65 polypeptide, but rather encode a fragment or fragments of a PtlP-50 polypeptide or PtlP-65 polypeptide. These polynucleotides can be used to express a functional PtlP-50 polypeptide or PtlP-65 polypeptide through a mechanism involving splicing, where splicing can occur at the level of polynucleotide (e.g., intron/exon) and/or polypeptide (e.g., intein/extein). This can be useful, for example, in controlling expression of pesticidal activity, since a functional pesticidal polypeptide will only be expressed if all required fragments are expressed in an environment that permits splicing processes to generate functional product. In another example, introduction of one or more insertion sequences into a polynucleotide can facilitate recombination with a low homology polynucleotide; use of an intron or intein for the insertion sequence facilitates the removal of the intervening sequence, thereby restoring function of the encoded variant.

[0079] Nucleic acid molecules that are fragments of these nucleic acid sequences encoding PtlP-50 polypeptides or PtlP-65 polypeptides are also encompassed by the disclosure. "Fragment" as used herein refers to a portion of the nucleic acid sequence encoding a PtlP-50 polypeptide or PtlP-65 polypeptide. A fragment of a nucleic acid sequence may encode a biologically active portion of a PtlP-50 polypeptide or PtlP-65 polypeptide or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. Nucleic acid molecules that are fragments of a nucleic acid sequence encoding a PtlP-50 polypeptide or PtlP-65 polypeptide comprise at least about 150, 180, 210, 240, 270, 300, 330 or 360, contiguous nucleotides or up to the number of nucleotides present in a full-length nucleic acid sequence encoding a PtlP-50 polypeptide or PtlP-65 polypeptide disclosed herein, depending upon the intended use. "Contiguous nucleotides" is used herein to refer to nucleotide residues that are immediately adjacent to one another. Fragments of the nucleic acid sequences of the embodiments will encode protein fragments that retain the biological activity of the PtlP-50 polypeptide / PtlP-65 polypeptide and, hence, retain insecticidal activity. "Retains insecticidal activity" is used herein to refer to a polypeptide having at least about 10%, at least about 30%, at least about 50%, at least about 70%, 80%, 90%, 95% or higher of the insecticidal activity of the full-length PtlP-50Aa polypeptide

in combination with PtIP-65Aa polypeptide. In some embodiments, the insecticidal activity is Lepidoptera activity. In one embodiment, the insecticidal activity is against a Coleopteran species. In some embodiments, the insecticidal activity is against one or more insect pests of the corn rootworm complex: western corn rootworm, *Diabrotica virgifera*; northern corn rootworm, *D. barberi*; Southern corn rootworm or spotted cucumber beetle; *Diabrotica undecimpunctata howardi*, and the Mexican corn rootworm, *D. virgifera zea*. In one embodiment, the insecticidal activity is against a *Diabrotica* species.

[0080] In some aspects a fragment of a nucleic acid sequence encoding a PtIP-50 polypeptide encoding a biologically active portion of a protein will encode at least about 15, 20, 30, 50, 75, 100, 125, contiguous amino acids or up to the total number of amino acids present in a full-length PtIP-50 polypeptide of the embodiments. In some aspects, the fragment is an N-terminal and/or a C-terminal truncation of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or more amino acids from the N-terminus and/or C-terminus, e.g., by proteolysis, insertion of a start codon, deletion of the codons encoding the deleted amino acids with the concomitant insertion of a stop codon or by insertion of a stop codon in the coding sequence. In some aspects, the fragments encompassed herein result from the removal of the N-terminal 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more amino acids from the N-terminus, e.g., by proteolysis or by insertion of a start codon in the coding sequence. In some aspects, the fragments encompassed herein result from the removal of the N-terminal 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 amino acids, e.g., by proteolysis or by insertion of a start codon in the coding sequence.

[0081] In some embodiments a PtIP-50 polypeptide is encoded by a nucleic acid sequence sufficiently homologous. "Sufficiently homologous" is used herein to refer to an amino acid or nucleic acid sequence that has at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence homology compared to a reference sequence using one of the alignment programs described herein using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding homology of proteins encoded by two nucleic acid sequences by taking into account codon degeneracy, amino acid similarity, and reading frame positioning. In some aspects the sequence homology is against the full length sequence of the polynucleotide encoding a PtIP-50 polypeptide or against the full length sequence of a PtIP-50 polypeptide. In some embodiments the PtIP-50 polypeptide has at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity. In some embodiments the sequence identity is calculated using ClustalW algorithm in the ALIGNX® module of the Vector NTI® Program Suite (Invitrogen Corporation, Carlsbad, Calif.) with all default parameters. In some embodiments the sequence identity is across the entire length of polypeptide calculated using ClustalW algorithm in the ALIGNX module of the Vector NTI Program Suite (Invitrogen Corporation, Carlsbad, Calif.) with all default parameters.

[0082] To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity=number of identical positions/total number of positions (e.g., overlapping positions)×100). In one embodiment, the two sequences are the same length. In another embodiment, the comparison is across the entirety of the reference sequence. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

[0083] The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, (1990) Proc. Natl. Acad. Sci. USA 87:2264, modified as in Karlin and Altschul, (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the BLASTN and BLASTX programs of Altschul, et al., (1990) J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the BLASTN program, score=100, wordlength=12, to obtain nucleic acid sequences homologous to pesticidal nucleic acid molecules of the embodiments. BLAST protein searches can be performed with the BLASTX program, score=50, wordlength=3, to obtain amino acid sequences homologous to pesticidal protein molecules of the embodiments. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul, et al., (1997) Nucleic Acids Res. 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. See, Altschul, et al., (1997) *supra*. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., BLASTX and BLASTN) can be used. Alignment may also be performed manually by inspection.

[0084] Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the ClustalW algorithm (Higgins, et al., (1994) Nucleic Acids Res. 22:4673-4680). ClustalW compares sequences and aligns the entirety of the amino acid or DNA sequence, and thus can provide data about the sequence conservation of the entire amino acid sequence. The ClustalW algorithm is used in several commercially available DNA/amino acid analysis software packages, such as the ALIGNX® module of the Vector NTI® Program Suite (Invitrogen Corporation, Carlsbad,

Calif.). After alignment of amino acid sequences with ClustalW, the percent amino acid identity can be assessed. A non-limiting example of a software program useful for analysis of ClustalW alignments is GENEDOC™. GENEDOC™ (Karl Nicholas) allows assessment of amino acid (or DNA) similarity and identity between multiple proteins. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) CABIOS 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG Wisconsin Genetics Software Package, Version 10 (available from Accelrys, Inc., 9685 Scranton Rd., San Diego, Calif., USA). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[0085] Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Needleman and Wunsch, (1970) J. Mol. Biol. 48(3):443-453, used GAP Version 10 software to determine sequence identity or similarity using the following default parameters: % identity and % similarity for a nucleic acid sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmpii scoring matrix; % identity or % similarity for an amino acid sequence using GAP weight of 8 and length weight of 2, and the BLOSUM62 scoring program. Equivalent programs may also be used. "Equivalent program" is used herein to refer to any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

[0086] The aspects also encompass nucleic acid molecules encoding PtIP-50 polypeptide variants. "Variants" of the PtIP-50 polypeptide encoding nucleic acid sequences include those sequences that encode the PtIP-50 polypeptides disclosed herein but that differ conservatively because of the degeneracy of the genetic code as well as those that are sufficiently identical as discussed above. Naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleic acid sequences also include synthetically derived nucleic acid sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the PtIP-50 polypeptides disclosed as discussed below.

[0087] The present disclosure provides isolated or recombinant polynucleotides that encode any of the PtIP-50 polypeptides disclosed herein. Those having ordinary skill in the art will readily appreciate that due to the degeneracy of the genetic code, a multitude of nucleotide sequences encoding PtIP-50 polypeptides of the present disclosure exist. Table 1 is a codon table that provides the synonymous codons for each amino acid. For example, the codons AGA, AGG, CGA, CGC, CGG, and CGU all encode the amino acid arginine. Thus, at every position in the nucleic acids of the disclosure where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide. It is understood that U in an RNA sequence corresponds to T in a DNA sequence.

Table 1

Alanine	Ala	GCA	GCC	GCG	GC		
Cysteine	Cys	UGC	UGU				
Aspartic acid	Asp	GAC	GA				
Glutamic acid	Glu	GAA	GAG				
Phenylalanine	Phe	UUC	UUU				
Glycine	Gly	GGA	GGC	GGG	GGU		
Histidine	His	CAC	CAU				
Isoleucine	Ile	AUA	AUC	AUU			
Lysine	Lys	AAA	AAG				
Leucine	Leu	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	AUG					
Asparagine	Asn	AAC	AAU				
Proline	Pro	CCA	CCC	CCG	CCU		
Glutamine	Gln	CAA	CAG				
Arginine	Arg	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	ACA	ACC	ACG	ACU		
Valine	Val	GUA	GUC	GUG	UU		
Tryptophan	Trp	UGG					
Tyrosine	Tyr	UAC	UAU				

[0088] The skilled artisan will further appreciate that changes can be introduced by mutation of the nucleic acid sequences thereby leading to changes in the amino acid sequence of the encoded PtIP-50 polypeptides, without altering the biological activity of the proteins. Thus, variant nucleic acid molecules can be created by introducing one or more nucleotide substitutions, additions and/or deletions into the corresponding nucleic acid sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleic acid sequences are also encompassed by the present disclosure.

[0089] Alternatively, variant nucleic acid sequences can be made by introducing mutations randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for ability to confer pesticidal activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly, and the activity of the protein can be determined using standard assay techniques.

[0090] The polynucleotides of the disclosure and fragments thereof are optionally used as substrates for a variety of recombination and recursive recombination reactions, in addition to standard cloning methods as set forth in, e.g., Ausubel, Berger and Sambrook, i.e., to produce additional pesticidal polypeptide homologues and fragments thereof with desired properties. A variety of such reactions are known, including those developed by the inventors and their co-workers. Methods for producing a variant of any nucleic acid listed herein comprising recursively recombining such polynucleotide with a second (or more) polynucleotide, thus forming a library of variant polynucleotides are also embodiments of the disclosure, as are the libraries produced, the cells comprising the libraries and any recombinant polynucleotide produced by such methods. Additionally, such methods optionally comprise selecting a variant polynucleotide from such libraries based on pesticidal activity, as is wherein such recursive recombination is done in vitro or in vivo.

[0091] A variety of diversity generating protocols, including nucleic acid recursive recombination protocols are available and fully described in the art. The procedures can be used separately, and/or in combination to produce one or more variants of a nucleic acid or set of nucleic acids, as well as variants of encoded proteins. Individually and collectively, these procedures provide robust, widely applicable ways of generating diversified nucleic acids and sets of nucleic acids (including, e.g., nucleic acid libraries) useful, e.g., for the engineering or rapid evolution of nucleic acids, proteins, pathways, cells and/or organisms with new and/or improved characteristics.

[0092] While distinctions and classifications are made in the course of the ensuing discussion for clarity, it will be appreciated that the techniques are often not mutually exclusive. Indeed, the various methods can be used singly or in combination, in parallel or in series, to access diverse sequence variants.

[0093] The result of any of the diversity generating procedures described herein can be the generation of one or more nucleic acids, which can be selected or screened for nucleic acids with or which confer desirable properties or that encode proteins with or which confer desirable properties. Following diversification by one or more of the methods herein or otherwise available to one of skill, any nucleic acids that are produced can be selected for a desired activity or property, e.g. pesticidal activity or, such activity at a desired pH, etc. This can include identifying any activity that can be detected, for example, in an automated or automatable format, by any of the assays in the art, see, e.g., discussion of screening of insecticidal activity, *infra*. A variety of related (or even unrelated) properties can be evaluated, in serial or in parallel, at the discretion of the practitioner.

[0094] Descriptions of a variety of diversity generating procedures for generating modified nucleic acid sequences, e.g., those coding for polypeptides having pesticidal activity or fragments thereof, are found in the following publications and the references cited therein: Soong, et al., (2000) *Nat Genet* 25(4):436-439; Stemmer, et al., (1999) *Tumor Targeting* 4:1-4; Ness, et al., (1999) *Nat Biotechnol* 17:893-896; Chang, et al., (1999) *Nat Biotechnol* 17:793-797; Minshull and Stemmer, (1999) *Curr Opin Chem Biol* 3:284-290; Christians, et al., (1999) *Nat Biotechnol* 17:259-264; Cramer, et al., (1998) *Nature* 391:288-291; Cramer, et al., (1997) *Nat Biotechnol* 15:436-438; Zhang, et al., (1997) *PNAS USA* 94:4504-4509; Patten, et al., (1997) *Curr Opin Biotechnol* 8:724-733; Cramer, et al., (1996) *Nat Med* 2:100-103; Cramer, et al., (1996) *Nat Biotechnol* 14:315-319; Gates, et al., (1996) *J Mol Biol* 255:373-386; Stemmer, (1996) "Sexual PCR and Assembly PCR" In: *The Encyclopedia of Molecular Biology*. VCH Publishers, New York. pp. 447-457; Cramer and Stemmer, (1995) *BioTechniques* 18:194-195; Stemmer, et al., (1995) *Gene*, 164:49-53; Stemmer, (1995) *Science* 270:1510; Stemmer, (1995) *Bio/Technology* 13:549-553; Stemmer, (1994) *Nature* 370:389-391 and Stemmer, (1994) *PNAS USA* 91:10747-10751.

[0095] Mutational methods of generating diversity include, for example, site-directed mutagenesis (Ling, et al., (1997) *Anal Biochem* 254(2):157-178; Dale, et al., (1996) *Methods Mol Biol* 57:369-374; Smith, (1985) *Ann Rev Genet* 19:423-462; Botstein and Shortle, (1985) *Science* 229:1193-1201; Carter, (1986) *Biochem J* 237:1-7 and Kunkel, (1987) "The efficiency of oligonucleotide directed mutagenesis" in *Nucleic Acids & Molecular Biology* (Eckstein and Lilley, eds., Springer Verlag, Berlin)); mutagenesis using uracil containing templates (Kunkel, (1985) *PNAS USA* 82:488-492; Kunkel, et al., (1987) *Methods Enzymol* 154:367-382 and Bass, et al., (1988) *Science* 242:240-245); oligonucleotide-directed mutagenesis (Zoller and Smith, (1983) *Methods Enzymol* 100:468-500; Zoller and Smith, (1987) *Methods Enzymol* 154:329-350 (1987); Zoller and Smith, (1982) *Nucleic Acids Res* 10:6487-6500), phosphorothioate-modified DNA mutagenesis (Taylor, et al., (1985) *Nucl Acids Res* 13:8749-8764; Taylor, et al., (1985) *Nucl Acids Res* 13:8765-8787

(1985); Nakamaye and Eckstein, (1986) Nucl Acids Res 14:9679-9698; Sayers, et al., (1988) Nucl Acids Res 16:791-802 and Sayers, et al., (1988) Nucl Acids Res 16:803-814); mutagenesis using gapped duplex DNA (Kramer, et al., (1984) Nucl Acids Res 12:9441-9456; Kramer and Fritz, (1987) Methods Enzymol 154:350-367; Kramer, et al., (1988) Nucl Acids Res 16:7207 and Fritz, et al., (1988) Nucl Acids Res 16:6987-6999).

5 **[0096]** Additional suitable methods include point mismatch repair (Kramer, et al., (1984) Cell 38:879-887), mutagenesis using repair-deficient host strains (Carter, et al., (1985) Nucl Acids Res 13:4431-4443 and Carter, (1987) Methods in Enzymol 154:382-403), deletion mutagenesis (Eghtedarzadeh and Henikoff, (1986) Nucl Acids Res 14:5115), restriction-selection and restriction-purification (Wells, et al., (1986) Phil Trans R Soc Lond A 317:415-423), mutagenesis by total gene synthesis (Nambiar, et al., (1984) Science 223:1299-1301; Sakamar and Khorana, (1988) Nucl Acids Res 14:6361-6372; Wells, et al., (1985) Gene 34:315-323 and Grundstrom, et al., (1985) Nucl Acids Res 13:3305-3316), double-strand break repair (Mandecki, (1986) PNAS USA, 83:7177-7181 and Arnold, (1993) Curr Opin Biotech 4:450-455). Additional details on many of the above methods can be found in Methods Enzymol Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis methods.

10 **[0097]** Additional details regarding various diversity generating methods can be found in the following US Patents, PCT Publications and Applications and EPO publications: US Patent Number 5,723,323, US Patent Number 5,763,192, US Patent Number 5,814,476, US Patent Number 5,817,483, US Patent Number 5,824,514, US Patent Number 5,976,862, US Patent Number 5,605,793, US Patent Number 5,811,238, US Patent Number 5,830,721, US Patent Number 5,834,252, US Patent Number 5,837,458, WO 1995/22625, WO 1996/33207, WO 1997/20078, WO 1997/35966, WO 1999/41402, WO 1999/41383, WO 1999/41369, WO 1999/41368, EP 752008, EP 0932670, WO 1999/23107, WO 20 1999/21979, WO 1998/31837, WO 1998/27230, WO 1998/27230, WO 2000/00632, WO 2000/09679, WO 1998/42832, WO 1999/29902, WO 1998/41653, WO 1998/41622, WO 1998/42727, WO 2000/18906, WO 2000/04190, WO 2000/42561, WO 2000/42559, WO 2000/42560, WO 2001/23401 and PCT/US01/06775.

25 **[0098]** The nucleotide sequences of the embodiments can also be used to isolate corresponding sequences from ferns or other primitive plants, particularly a *Asplenium*, *Polypodium Adiantum*, *Platyserium*, *Nephrolepis*, *Ophioglossum*, *Colysis*, *Bolbitis*, *Blechnum*, *Selaginella*, *Lycopodium*, and *Huperzia* species. In this manner, methods such as PCR, and hybridization can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences that are selected based on their sequence identity to the entire sequences set forth herein or to fragments thereof are encompassed by the embodiments. Such sequences include sequences that are orthologs of the disclosed sequences. The term "orthologs" refers to genes derived from a common ancestral gene and which are found in different species as a result of speciation. Genes found in different species are considered orthologs when their nucleotide sequences and/or their encoded protein sequences share substantial identity as defined elsewhere herein. Functions of orthologs are often highly conserved among species.

30 **[0099]** In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any organism of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook, et al., (1989) Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York), hereinafter "Sambrook". See also, Innis, et al., eds. (1990) PCR Protocols: A Guide to Methods and Applications (Academic Press, New York); Innis and Gelfand, eds. (1995) PCR Strategies (Academic Press, New York); and Innis and Gelfand, eds. (1999) PCR Methods Manual (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, and partially-mismatched primers.

35 **[0100]** To identify potential PtIP-50 polypeptides and/or PtIP-65 polypeptides from fern or moss collections, the fern or moss cell lysates can be screened with antibodies generated against a PtIP-50 polypeptides and/or PtIP-65 polypeptides using Western blotting and/or ELISA methods. This type of assays can be performed in a high throughput fashion. Positive samples can be further analyzed by various techniques such as antibody based protein purification and identification. Methods of generating antibodies are well known in the art as discussed infra.

40 **[0101]** Alternatively, mass spectrometry based protein identification method can be used to identify homologs of PtIP-50 polypeptides and/or PtIP-65 polypeptides using protocols in the literatures (Scott Patterson, (1998), 10.22, 1-24, Current Protocol in Molecular Biology published by John Wiley & Son Inc). Specifically, LC-MS/MS based protein identification method is used to associate the MS data of given cell lysate or desired molecular weight enriched samples (excised from SDS-PAGE gel of relevant molecular weight bands to PtIP-50 polypeptides and/or PtIP-65 polypeptides) with sequence information of PtIP-50 polypeptides and/or PtIP-65 polypeptides and its homologs. Any match in peptide sequences indicates the potential of having the homologous proteins in the samples. Additional techniques (protein purification and molecular biology) can be used to isolate the protein and identify the sequences of the homologs.

45 **[0102]** In hybridization methods, all or part of the pesticidal nucleic acid sequence can be used to screen cDNA or genomic libraries. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook and Russell, (2001), *supra*. The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments or other oligonucleotides and may be labeled with a detectable group such as 32P or

any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme or an enzyme co-factor. Probes for hybridization can be made by labeling synthetic oligonucleotides based on the known PtIP-50 polypeptide or PtIP-65 polypeptide-encoding nucleic acid sequence disclosed herein. Degenerate primers designed on the basis of conserved nucleotides or amino acid residues in the nucleic acid sequence or encoded amino acid sequence can additionally be used. The probe typically comprises a region of nucleic acid sequence that hybridizes under stringent conditions to at least about 12, at least about 25, at least about 50, 75, 100, 125, 150, 175 or 200 consecutive nucleotides of nucleic acid sequence encoding a PtIP-50 polypeptide or PtIP-65 polypeptide of the disclosure or a fragment or variant thereof. Methods for the preparation of probes for hybridization are generally known in the art and are disclosed in Sambrook and Russell, (2001), *supra*.

[0103] For example, an entire nucleic acid sequence, encoding a PtIP-50 polypeptide and/or PtIP-65 polypeptide, disclosed herein or one or more portions thereof may be used as a probe capable of specifically hybridizing to corresponding nucleic acid sequences encoding PtIP-50 polypeptide or PtIP-65 polypeptide-like sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique and are preferably at least about 10 nucleotides in length or at least about 20 nucleotides in length. Such probes may be used to amplify corresponding pesticidal sequences from a chosen organism by PCR. This technique may be used to isolate additional coding sequences from a desired organism or as a diagnostic assay to determine the presence of coding sequences in an organism. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook, et al., (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

[0104] Hybridization of such sequences may be carried out under stringent conditions. "Stringent conditions" or "stringent hybridization conditions" is used herein to refer to conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

[0105] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C., and a wash in 1× to 2×SSC (20×SSC=3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C., and a wash in 0.5× to 1×SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C., and a wash in 0.1×SSC at 60 to 65°C. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

[0106] Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5^\circ\text{C.} + 16.6 (\log M) + 0.41 (\% \text{ GC}) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, % GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3 or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9 or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, (1993) *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, N.Y.); and Ausubel, et al., eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See, Sambrook, et al., (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press,

Cold Spring Harbor, N.Y.).

Proteins and Variants and Fragments Thereof

- 5 **[0107]** PtIP-50 polypeptides and PtIP-65 polypeptides are also encompassed by the disclosure. "Pteridophyta Insecticidal Protein-50" "PtIP-50 polypeptide", and "PtIP-50 protein" as used herein interchangeably refers to a polypeptide having insecticidal activity including but not limited to insecticidal activity against one or more insect pests of the Lepidoptera and/or Coleoptera orders, and is sufficiently homologous to the protein. A variety of PtIP-50 polypeptides are contemplated. Sources of PtIP-50 polypeptides or related proteins are fern species selected from but not limited to
- 10 *Asplenium australasicum*, *Asplenium nidus*, *Asplenium x kenzoii* Sa. Kurata, *Polypodium musifolium*, *Polypodium punctatum* 'Serratum', *Adiantum pedatum* L., *Platyserium bifurcatum*, *Nephrolepis falcata*, *Colysis wrightii* 'Monstifera', *Colysis wrightii*, *Bolbitis cladorrhizans*, and *Blechnum brasiliense* 'Crispum'. "Pteridophyta Insecticidal Protein-65" "PtIP-65 polypeptide", and "PtIP-65 protein" as used herein interchangeably refers to a polypeptide having insecticidal activity including but not limited to insecticidal activity against one or more insect pests of the Lepidoptera and/or Coleoptera
- 15 orders, and is sufficiently homologous to the protein. A variety of PtIP-65 polypeptides are contemplated. Sources of PtIP-65 polypeptides or related proteins are fern species selected from but not limited to: *Asplenium australasicum*, *Asplenium x kenzoii* Sa. Kurata, *Polypodium musifolium*, *Polypodium punctatum* 'Serratum', *Adiantum pedatum* L., *Platyserium bifurcatum*, *Nephrolepis falcata*, *Ophioglossum pendulum*, *Colysis wrightii* 'Monstifera', *Colysis wrightii*, *Selaginella kraussiana* 'Variegata', *Selaginella victoriae*, *Lycopodium phlehmaria*, and *Huperzia salvinoides*.
- 20 **[0108]** "Sufficiently homologous" is used herein to refer to an amino acid sequence that has at least about 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence homology compared to a reference sequence using one of the alignment programs described herein using standard parameters. In some aspects the
- 25 sequence homology is against the full length sequence of a PtIP-50 polypeptide. In some embodiments the PtIP-50 polypeptide has at least about 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity. In some embodiments the sequence homology is against the full length sequence a PtIP-65 polypeptide. In
- 30 some aspects the PtIP-65 polypeptide has at least about 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding homology of proteins taking into account amino acid similarity. In some embodiments the
- 35 sequence identity is calculated using ClustalW algorithm in the ALIGNX® module of the Vector NTI® Program Suite (Invitrogen Corporation, Carlsbad, Calif.) with all default parameters. In some embodiments the sequence identity is across the entire length of polypeptide calculated using ClustalW algorithm in the ALIGNX® module of the Vector NTI® Program Suite (Invitrogen Corporation, Carlsbad, Calif.) with all default parameters.
- [0109]** As used herein, the terms "protein," "peptide molecule," or "polypeptide" includes any molecule that comprises
- 40 five or more amino acids. It is well known in the art that protein, peptide or polypeptide molecules may undergo modification, including post-translational modifications, such as, but not limited to, disulfide bond formation, glycosylation, phosphorylation or oligomerization. Thus, as used herein, the terms "protein," "peptide molecule" or "polypeptide" includes any protein that is modified by any biological or non-biological process. The terms "amino acid" and "amino acids" refer to all naturally occurring L-amino acids.
- 45 **[0110]** A "recombinant protein" is used herein to refer to a protein that is no longer in its natural environment, for example in vitro or in a recombinant bacterial or plant host cell. A PtIP-50 polypeptide or PtIP-65 polypeptide that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10% or 5% (by dry weight) of non-pesticidal protein (also referred to herein as a "contaminating protein").
- [0111]** "Fragments" or "biologically active portions" include polypeptide fragments comprising amino acid sequences
- 50 sufficiently identical to a PtIP-50 polypeptide or PtIP-65 polypeptide and that exhibit insecticidal activity. "Fragments" or "biologically active portions" of PtIP-50 polypeptides includes fragments comprising amino acid sequences sufficiently identical, wherein the PtIP-50 polypeptide, in combination with a PtIP-65 polypeptide, has insecticidal activity. "Fragments" or "biologically active portions" of PtIP-65 polypeptides includes fragments comprising amino acid sequences sufficiently identical, wherein the PtIP-65 polypeptide, in combination with a PtIP-50 polypeptide, has insecticidal activity.
- 55 Such biologically active portions can be prepared by recombinant techniques and evaluated for insecticidal activity. In some embodiments, the PtIP-50 polypeptide fragment is an N-terminal and/or a C-terminal truncation of at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or more amino acids from the N-terminus and/or C-terminus e.g., by proteolysis, by insertion of a start codon, by deletion of the codons

encoding the deleted amino acids and concomitant insertion of a start codon, and/or insertion of a stop codon.

[0112] In some embodiments, the PtP-50 polypeptide fragments encompassed herein result from the removal of the N-terminal 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more amino acids, e.g., by proteolysis or by insertion of a start codon, by deletion of the codons encoding the deleted amino acids and concomitant insertion of a start codon.

[0113] "Variants" as used herein refers to proteins or polypeptides having an amino acid sequence that is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the parental amino acid sequence.

PtIP-50 polypeptides

[0114] In some embodiments a PtIP-50 polypeptide comprises an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70 or more amino acid substitutions compared to the native amino acid at the corresponding position.

[0115] In some embodiments the sequence identity is across the entire length of the polypeptide calculated using ClustalW algorithm in the ALIGNX® module of the Vector NTI® Program Suite (Invitrogen Corporation, Carlsbad, Calif.) with all default parameters.

[0116] In some embodiments a PtIP-50 polypeptide comprises an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 amino acid substitutions, in any combination, compared to the native amino acid at the corresponding position.

[0117] In some embodiments a PtIP-50 polypeptide comprises an amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 or 29 amino acid substitutions, in any combination, compared to the native amino acid at the corresponding position.

[0118] In some embodiments the nucleic acid molecule encoding the PtIP-50 polypeptide is derived from a fern species in the Division *Pteridophyta*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Psilotales*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*, Family *Psilotaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*, Family *Ophioglossaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Genus *Ophioglossum* L., *Botrychium*, *Botrypus*, *Helminthostachys*, *Ophioderma*, *Cheiroglossa*, *Sceptridium* or *Mankyua*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Genus *Ophioglossum* L. is selected from but not limited to *Ophioglossum californicum*, *Ophioglossum coriaceum*, *Ophioglossum costatum*, *Ophioglossum crotalophoroides*, *Ophioglossum engelmannii*, *Ophioglossum falcatum*, *Ophioglossum gomezianum*, *Ophioglossum gramineum*, *Ophioglossum kawamurae*, *Ophioglossum lusitanicum*, *Ophioglossum namegatae*, *Ophioglossum nudicaule*, *Ophioglossum palmatum*, *Ophioglossum parvum*, *Ophioglossum pedunculatum*, *Ophioglossum pendulum*, *Ophioglossum petiolatum*, *Ophioglossum pusillum*, *Ophioglossum reticulatum*, *Ophioglossum richardsiae*, *Ophioglossum thermale*, and *Ophioglossum vulgatum*.

[0119] In some embodiments the PtIP-50 polypeptide is derived from a species in the Class *Polypodiopsida*/*Pteridopsida*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Osmundales* (royal ferns); Family *Osmundaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Hymenophyllales* (filmy ferns and bristle ferns); Family *Hymenophyllaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Gleicheniales*; Family *Gleicheniaceae*, Family *Dipteridaceae* or Family *Matoniaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Schizaeales*; Family *Lygodiaceae*, Family *Anemiaceae* or Family *Schizaeaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Salviniales*; Family *Marsileaceae* or Family *Salviniaceae*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Cyatheaales*; Family *Thyrsopteridaceae*, Family *Loxosomataceae*, Family *Culcitaceae*, Family *Plagiogyriaceae*, Family *Cibotiaceae*, Family *Cyatheaceae*, Family *Dicksoniaceae* or Family *Metaxyaceae*.

[0120] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Polypodiales*; Family *Lindsaeaceae*, Family *Saccolomataceae*, Family *Cystodiaceae*, Family *Dennstaedtiaceae*, Family *Pteridaceae*, Family *Aspleniaceae*, Family *Thelypteridaceae*, Family *Woodsiaceae*, Family *Onocleaceae*, Family *Blechnaceae*, Family *Dryopteridaceae*, Family *Lomariopsidaceae*, Family *Tectariaceae*, Family *Oleandraceae*, Family *Davalliaceae* or Family *Polypodiaceae*.

[0121] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order *Polypodiales*, Family *Pteridaceae*, Genus *Adiantaceae* selected from but not limited to *Adiantum aethiopicum*, *Adiantum aleuticum*, *Adiantum bonatianum*, *Adiantum cajennense*, *Adiantum capillus-junonis*, *Adiantum capillus-veneris*, *Adiantum caudatum*, *Adian-*

tum chienii, *Adiantum chilense*, *Adiantum cuneatum*, *Adiantum cunninghamii*, *Adiantum davidii*, *Adiantum diaphanum*, *Adiantum edentulum*, *Adiantum edgeworthii*, *Adiantum excisum*, *Adiantum fengianum*, *Adiantum fimbriatum*, *Adiantum flabellulatum*, *Adiantum formosanum*, *Adiantum formosum*, *Adiantum fulvum*, *Adiantum gravesii*, *Adiantum hispidulum*, *Adiantum induratum*, *Adiantum jordanii*, *Adiantum juxtapositum*, *Adiantum latifolium*, *Adiantum leveillei*, *Adiantum lianxianense*, *Adiantum malesianum*, *Adiantum mariesii*, *Adiantum monochlamys*, *Adiantum myriosorum*, *Adiantum obliquum*, *Adiantum ogasawarensis*, *Adiantum pedatum*, *Adiantum pentadactylon*, *Adiantum peruvianum*, *Adiantum philippense*, *Adiantum princeps*, *Adiantum pubescens*, *Adiantum raddianum*, *Adiantum reniforme*, *Adiantum roborowskii*, *Adiantum serratodentatum*, *Adiantum sinicum*, *Adiantum soboliferum*, *Adiantum subcordatum*, *Adiantum tenerum*, *Adiantum terminatum*, *Adiantum tetraphyllum*, *Adiantum venustum*, *Adiantum viridescens*, and *Adiantum viridimontanum*.

[0122] In some embodiments the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Aspleniaceae, Genus *Asplenium* L. In some embodiments the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Aspleniaceae, Genus *Asplenium* L selected from but not limited to *Asplenium abbreviatum*, *Asplenium abrotanoides*, *Asplenium abscissum* var. *subaequilaterale*, *Asplenium abscissum*, *Asplenium achilleifolium*, *Asplenium acuminatum*, *Asplenium adiantifrons*, *Asplenium adiantoides*, *Asplenium adiantoides* var. *squamulosum*, *Asplenium adiantum-nigrum* L., *Asplenium adiantum-nigrum* var. *adiantum-nigrum*, *Asplenium adiantum-nigrum* var. *yuanum*, *Asplenium adnatum*, *Asplenium aethiopicum*, *Asplenium affine*, *Asplenium affine* var. *affine*, *Asplenium affine* var. *gilpinae*, *Asplenium affine* var. *mettenii*, *Asplenium affine* var. *pecten*, *Asplenium africanum*, *Asplenium afzeli*, *Asplenium aitchisonii*, *Asplenium alatulum*, *Asplenium alatulum*, *Asplenium alfredii*, *Asplenium altajense*, *Asplenium amabile*, *Asplenium ambohitantelense*, *Asplenium anceps* var. *proliferum*, *Asplenium andapense*, *Asplenium andersonii*, *Asplenium angustatum*, *Asplenium angustum*, *Asplenium anisophyllum*, *Asplenium annetii*, *Asplenium antiquum*, *Asplenium antrophyoides*, *Asplenium apertum*, *Asplenium apogamum*, *Asplenium aquaticum*, *Asplenium arboreum*, *Asplenium arcanum*, *Asplenium arcuatum*, *Asplenium argentinum*, *Asplenium argutum*, *Asplenium aspidiiforme*, *Asplenium aspidioides*, *Asplenium asterolepis*, *Asplenium auricularium* var. *acutidens*, *Asplenium auricularium* var. *subintegerrimum*, *Asplenium auriculatum*, *Asplenium auriculatum* var. *aequilaterale*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *nana*, *Asplenium auritum*, *Asplenium auritum* var. *auriculatum*, *Asplenium auritum* var. *auritum*, *Asplenium auritum* var. *bipinnatifidum*, *Asplenium auritum* var. *bipinnatisectum*, *Asplenium auritum* var. *davallioides*, *Asplenium auritum* var. *macilentum*, *Asplenium auritum* var. *rigidum*, *Asplenium auritum* var. *subsimplax*, *Asplenium austrochinense*, *Asplenium ayopayense*, *Asplenium badinii*, *Asplenium balense*, *Asplenium ballivianii*, *Asplenium bangii*, *Asplenium bangii*, *Asplenium barbaense*, *Asplenium barclayanum*, *Asplenium barkamense*, *Asplenium barteri*, *Asplenium basisopicum*, *Asplenium bicrenatum*, *Asplenium bifrons*, *Asplenium bipartitum*, *Asplenium blastophorum*, *Asplenium blepharodes*, *Asplenium blepharophorum*, *Asplenium boiteaui*, *Asplenium bolivianum*, *Asplenium boltonii*, *Asplenium borealichinense*, *Asplenium bradei*, *Asplenium bradeorum*, *Asplenium bradleyi*, *Asplenium brausei*, *Asplenium breedlovei*, *Asplenium buettneri*, *Asplenium buettneri* var. *hildebrandtii*, *Asplenium bulbiferum*, *Asplenium bullatum* var. *bullatum*, *Asplenium bullatum* var. *shikokianum*, *Asplenium bullatum*, *Asplenium cancellatum*, *Asplenium capillipes*, *Asplenium cardiophyllum* (Hance), *Asplenium caripense*, *Asplenium carvalhoanum*, *Asplenium castaneoviride*, *Asplenium castaneum*, *Asplenium caudatum*, *Asplenium celtidifolium* (Kunze), *Asplenium ceratolepis*, *Asplenium changputungense*, *Asplenium chaseanum*, *Asplenium cheilosorum*, *Asplenium chengkouense*, *Asplenium chihuahuense*, *Asplenium chimantae*, *Asplenium chimborazense*, *Asplenium chingianum*, *Asplenium chlorophyllum*, *Asplenium chondrophyllum*, *Asplenium cicutarium*, *Asplenium cicutarium* var. *paleaceum*, *Asplenium cirrhatum*, *Asplenium cladolepton*, *Asplenium claussenii*, *Asplenium coenobiale*, *Asplenium commutatum*, *Asplenium congestum*, *Asplenium conquisitum*, *Asplenium consimile*, *Asplenium contiguum*, *Asplenium contiguum* var. *hirtulum*, *Asplenium corderoi*, *Asplenium cordovense*, *Asplenium coriaceum*, *Asplenium coriifolium*, *Asplenium correardii*, *Asplenium costale*, *Asplenium costale* var. *robustum*, *Asplenium cowanii*, *Asplenium crenuloserrulatum*, *Asplenium crenulatum*, *Asplenium crinicaule*, *Asplenium crinulosum*, *Asplenium cristatum*, *Asplenium cryptolepis* Fernald, *Asplenium cultrifolium* L., *Asplenium cuneatifforme*, *Asplenium cuneatum*, *Asplenium curvatum*, *Asplenium cuspidatum*, *Asplenium cuspidatum* var. *cuspidatum*, *Asplenium cuspidatum* var. *foeniculaceum*, *Asplenium cuspidatum* var. *triculum*, *Asplenium cuspidatum* var. *tripinnatum*, *Asplenium dalhousiae*, *Asplenium dareoides*, *Asplenium davallioides*, *Asplenium davisii*, *Asplenium debile*, *Asplenium debile*, *Asplenium decussatum*, *Asplenium delavayi*, *Asplenium delicatulum*, *Asplenium delicatulum* var. *cocosensis*, *Asplenium delitescens*, *Asplenium delitescens* X *laetum*, *Asplenium densum*, *Asplenium dentatum* L., *Asplenium dentatum* L., *Asplenium depauperatum*, *Asplenium deqenense*, *Asplenium diana*, *Asplenium difforme*, *Asplenium dilatatum*, *Asplenium dimidiatum*, *Asplenium dimidiatum* var. *boliviense*, *Asplenium diplazisorum*, *Asplenium dissectum*, *Asplenium distans*, *Asplenium divaricatum*, *Asplenium divergens*, *Asplenium divisissimum*, *Asplenium doederleinii*, *Asplenium donnell-smithii*, *Asplenium dregeanum*, *Asplenium dulongjiangense*, *Asplenium duplicatoserratum*, *Asplenium eatonii*, *Asplenium ebeneum*, *Asplenium ebenoides*, *Asplenium ecuadorensis*, *Asplenium eggertii*, *Asplenium emarginatum*, *Asplenium enatum*, *Asplenium ensiforme* fo. *bicuspe*, *Asplenium ensiforme* fo. *ensiforme*, *Asplenium ensiforme* fo. *stenophyllum*, *Asplenium ensiforme*, *Asplenium erectum* var. *erectum*, *Asplenium erectum* var. *gracile*, *Asplenium erectum* var. *usambarense*, *Asplenium erectum* var. *zeyheri*, & *Asplenium erosum* L., *Asplenium escaleroense*, *Asplenium esculentum*, *Asplenium eutecnum*, *Asplenium excelsum*, *Asplenium excisum*, *Asplenium exiguum*, *Asplenium extensum*,

Asplenium falcatum, *Asplenium falcinellum*, *Asplenium faurei*, *Asplenium feei*, *Asplenium fengyangshanense*, *Asplenium ferulaceum*, *Asplenium fibrillosum*, *Asplenium filix-femina*, *Asplenium finckii*, *Asplenium finlaysonianum*, *Asplenium flabellulatum*, *Asplenium flabellulatum var flabellulatum*, *Asplenium flabellulatum var. partitum*, *Asplenium flaccidum*, *Asplenium flavescens*, *Asplenium flavidum*, *Asplenium flexuosum*, *Asplenium fluminense*, *Asplenium foeniculaceum*, *Asplenium formosanum*, *Asplenium formosum var. carolinum*, *Asplenium formosum var. incultum*, *Asplenium formosum*, *Asplenium fournieri*, *Asplenium fragile*, *Asplenium fragile var. lomense*, *Asplenium fragrans*, *Asplenium fragrans var. foeniculaceum*, *Asplenium franconis var. gracile*, *Asplenium fraxinifolium*, *Asplenium friesiorum*, *Asplenium friesiorum var. nesophilum*, *Asplenium fugax*, *Asplenium fujianense*, *Asplenium furcatum*, *Asplenium furfuraceum*, *Asplenium fuscipes*, *Asplenium fuscopubescens*, *Asplenium galeottii*, *Asplenium gautieri*, *Asplenium gemmiferum*, *Asplenium gentryi*, *Asplenium geppii*, *Asplenium ghiesbreghtii*, *Asplenium gilliesii*, *Asplenium gilpinae*, *Asplenium glanduliserratum*, *Asplenium glenniei*, *Asplenium goldmannii*, *Asplenium gomezianum*, *Asplenium grande*, *Asplenium grandifolium*, *Asplenium grandifrons*, *Asplenium gregoriae*, *Asplenium griffithianum*, *Asplenium gulingense*, *Asplenium hainanense*, *Asplenium hallbergii*, *Asplenium hallei*, *Asplenium hallii*, *Asplenium hangzhouense*, *Asplenium haplophyllum*, *Asplenium harpeodes*, *Asplenium harpeodes var. glaucovirens*, *Asplenium harpeodes var. incisum*, *Asplenium harrisii Jenman*, *Asplenium harrisonii*, *Asplenium hastatum*, *Asplenium hebeiense*, *Asplenium hemionitideum*, *Asplenium hemitomum*, *Asplenium henryi*, *Asplenium herpetopteris*, *Asplenium herpetopteris var herpetopteris*, *Asplenium herpetopteris var. acutipinnata*, *Asplenium herpetopteris var. masoulae*, *Asplenium herpetopteris var. villosum*, *Asplenium hesperium*, *Asplenium heterochroum*, *Asplenium hians*, *Asplenium hians var. pallescens*, *Asplenium hoffmannii*, *Asplenium holophlebium*, *Asplenium hondoense*, *Asplenium horridum*, *Asplenium hostmannii*, *Asplenium humistratum*, *Asplenium hypomelas*, *Asplenium inaequilaterale*, *Asplenium incisum*, *Asplenium incurvatum*, *Asplenium indicum*, *Asplenium indicum var. indicum*, *Asplenium indicum var. yoshingagae*, *Asplenium induratum*, *Asplenium indusiatum*, *Asplenium inexpectatum*, *Asplenium insigne*, *Asplenium institicium*, *Asplenium insolitum*, *Asplenium integerrimum*, *Asplenium interjectum*, *Asplenium jamesonii*, *Asplenium jaundeense*, *Asplenium juglandifolium*, *Asplenium kangdingense*, *Asplenium kansuense*, *Asplenium kassneri*, *Asplenium kaulfussii*, *Asplenium kellermanii*, *Asplenium kentuckiense*, *Asplenium khullarii*, *Asplenium kiangsuense*, *Asplenium kunzeanum*, *Asplenium lacerum*, *Asplenium laciniatum*, *Asplenium laciniatum var. acutipinna*, *Asplenium laciniatum var. laciniatum*, *Asplenium laetum fo. minor*, *Asplenium laetum*, *Asplenium laetum var. incisoserratum*, *Asplenium lamprocaulon*, *Asplenium laserpitiifolium var. morrisonense*, *Asplenium lastii*, *Asplenium latedens*, *Asplenium latifolium*, *Asplenium laui*, *Asplenium laurentii*, *Asplenium leandrianum*, *Asplenium lechleri*, *Asplenium leiboense*, *Asplenium lepidorachis*, *Asplenium leptochlamys*, *Asplenium leptophyllum*, *Asplenium levyi*, *Asplenium lindbergii*, *Asplenium lindenii*, *Asplenium lineatum*, *Asplenium lividum*, *Asplenium lobatum*, *Asplenium lobulatum*, *Asplenium lokohoense*, *Asplenium longicauda*, *Asplenium longicaudatum*, *Asplenium longifolium*, *Asplenium longisorum*, *Asplenium longjiinense*, *Asplenium lorentzii*, *Asplenium loriceum*, *Asplenium loxogrammoides*, *Asplenium lugubre*, *Asplenium lunulatum*, *Asplenium lunulatum var. pteropus*, *Asplenium lushanense*, *Asplenium lydgatei*, *Asplenium macilentum*, *Asplenium macraei*, *Asplenium macrodictyon*, *Asplenium macrophlebium*, *Asplenium macrophyllum*, *Asplenium macropterum*, *Asplenium macrosorum*, *Asplenium macrotis*, *Asplenium macrurum*, *Asplenium mainlingense*, *Asplenium mangindranense*, *Asplenium mannii*, *Asplenium marginatum L.*, *Asplenium marojejense*, *Asplenium martianum*, *Asplenium matsumurae*, *Asplenium mauritiensis Lorence*, *Asplenium maximum*, *Asplenium, ii*, *Asplenium megalura*, *Asplenium megaphyllum*, *Asplenium meiotomum*, *Asplenium melanopus*, *Asplenium membranifolium*, *Asplenium meniscioides*, *Asplenium mesosorum*, *Asplenium mexicanum*, *Asplenium micropaleatum*, *Asplenium microtum*, *Asplenium mildbraedii*, *Asplenium mildei*, *Asplenium minimum*, *Asplenium minutum*, *Asplenium miradoreense*, *Asplenium miyunense*, *Asplenium mocceianum*, *Asplenium mocquersii*, *Asplenium modestum*, *Asplenium monanthes var. menziesii*, *Asplenium monanthes L.*, *Asplenium monanthes var monanthes*, *Asplenium monanthes var. castaneum*, *Asplenium monanthes var. wagneri*, *Asplenium monanthes var. yungense*, *Asplenium monodon*, *Asplenium montanum*, *Asplenium mosetenense*, *Asplenium moupinense*, *Asplenium mucronatum*, *Asplenium munchii*, *Asplenium muticum*, *Asplenium myapteron*, *Asplenium myriophyllu*, *Asplenium nakanoanum*, *Asplenium nanchuanense*, *Asplenium nemorale*, *Asplenium neolaserpitiifolium*, *Asplenium neomutijugum*, *Asplenium neovarians*, *Asplenium nesii*, *Asplenium nesioticum*, *Asplenium nidus L.*, *Asplenium nigricans*, *Asplenium niponicum*, *Asplenium normale*, *Asplenium normale var. angustum*, *Asplenium obesum*, *Asplenium oblongatum*, *Asplenium oblongifolium*, *Asplenium obovatum*, *Asplenium obscurum*, *Asplenium obscurum var. angustum*, *Asplenium obtusatum var. obtusatum*, *Asplenium obtusatum var. sphenoides*, *Asplenium obtusifolium L.*, *Asplenium obtusissimum*, *Asplenium obversum*, *Asplenium ochraceum*, *Asplenium oellgaardii*, *Asplenium ofeliae*, *Asplenium oldhami*, *Asplenium oligosorum*, *Asplenium olivaceum*, *Asplenium onopteris L.*, *Asplenium onustum*, *Asplenium ortegae*, *Asplenium otites*, *Asplenium palaciosii*, *Asplenium palmeri*, *Asplenium partitum*, *Asplenium parvisorum*, *Asplenium parvisculum*, *Asplenium parvulum*, *Asplenium patens*, *Asplenium paucifolium*, *Asplenium paucijugum*, *Asplenium paucivinosum*, *Asplenium pearcei*, *Asplenium pekinense*, *Asplenium pellucidum*, *Asplenium pendulum*, *Asplenium petiolulatum*, *Asplenium phyllitidis*, *Asplenium pimpinellifolium*, *Asplenium pinnatifidum*, *Asplenium pinnatum*, *Asplenium platyneuron*, *Asplenium platyneuron var. bacculum-rubrum*, *Asplenium platyneuron var. incisum*, *Asplenium platyphyllum*, *Asplenium plumbeum*, *Asplenium poloense*, *Asplenium polymeris*, *Asplenium polymorphum*, *Asplenium polyodon*, *Asplenium polyodon var. knudsenii*, *Asplenium polyodon var. nitidulum*, *Asplenium polyodon var. sectum*, *Asplenium*

polyodon var. *subcaudatum*, *Asplenium polyphyllum*, *Asplenium poolii*, *Asplenium poolii* fo. *simplex*, *Asplenium poolii* var. *linearipinnatum*, *Asplenium potosinum*, *Asplenium potosinum* var. *incisum*, *Asplenium praegracile*, *Asplenium praemorsum*, *Asplenium preussii*, *Asplenium pringleanum*, *Asplenium pringlei*, *Asplenium prionitis*, *Asplenium procerum*, *Asplenium progrediens*, *Asplenium projectum*, *Asplenium prolongatum*, *Asplenium propinquum*, *Asplenium protensum*,
5 *Asplenium pseudoangustum*, *Asplenium pseudoerectum*, *Asplenium pseudofontanum*, *Asplenium pseudolaserpitiifolium*, *Asplenium pseudonormale*, *Asplenium pseudopellucidum*, *Asplenium pseudopraemorsum*, *Asplenium pseudovarians*, *Asplenium pseudowilfordii*, *Asplenium pseudowrightii*, *Asplenium psilacrum*, *Asplenium pteropus*, *Asplenium pubirhizoma*, *Asplenium pulchellum*, *Asplenium pulchellum* var. *subhorizontale*, *Asplenium pulcherrimum*, *Asplenium pulicosum*, *Asplenium pulicosum* var. *maius*, *Asplenium pululahuae*, *Asplenium pumilum*, *Asplenium pumilum* var. *hymenophylloides*,
10 *Asplenium pumilum* var. *laciniatum*, *Asplenium purdieanum*, *Asplenium purpurascens*, *Asplenium pyramidatum*, *Asplenium qiujiangense*, *Asplenium quercicola*, *Asplenium quitense*, *Asplenium raddianum*, *Asplenium radiatum*, *Asplenium radicans* L., *Asplenium radicans*, *Asplenium radicans* var. *costaricense*, *Asplenium radicans* var. *partitum*, *Asplenium radicans* var. *radicans*, *Asplenium radicans* var. *uniseriale*, *Asplenium recumbens*, *Asplenium reflexum*, *Asplenium regulare* var. *latior*, *Asplenium repandulum*, *Asplenium repens*, *Asplenium repente*, *Asplenium resiliens*,
15 *Asplenium retusulum*, *Asplenium rhipidoneuron*, *Asplenium rhizophorum* L., *Asplenium rhizophyllum*, *Asplenium rhizophyllum* L., *Asplenium rhizophyllum* var. *proliferum*, *Asplenium rhomboideum*, *Asplenium rigidum*, *Asplenium riparium*, *Asplenium rivale*, *Asplenium rockii*, *Asplenium roemerianum*, *Asplenium roemerianum* var. *mindensis*, *Asplenium rosenstockianum*, *Asplenium rubinum*, *Asplenium ruizianum*, *Asplenium rusbyanum*, *Asplenium ruta-muraria* L., *Asplenium ruta-muraria* var. *cryptolepis*, *Asplenium rutaceum*, *Asplenium rutaceum* var. *disculiferum*, *Asplenium rutaefolium*, *Asplenium rutifolium*,
20 *Asplenium salicifolium* L., *Asplenium salicifolium* var. *aequilaterale*, *Asplenium salicifolium* var. *salicifolium*, *Asplenium sampsoni*, *Asplenium sanchezii*, *Asplenium sanderi*, *Asplenium sandersonii*, *Asplenium sanguinolentum*, *Asplenium sarelii*, *Asplenium sarelii* var. *magnum*, *Asplenium sarelii* var. *sarelii*, *Asplenium saxicola*, *Asplenium scalifolium*, *Asplenium scandicinum*, *Asplenium schizophyllum*, *Asplenium schkuhrii*, *Asplenium sciadophilum*, *Asplenium scolopendrium* L., *Asplenium scortechinii*, *Asplenium seileri*, *Asplenium semipinnatum*, *Asplenium septentrionale*,
25 *Asplenium serra*, *Asplenium serra* var. *imrayanum*, *Asplenium serratissimum*, *Asplenium serratum* L., *Asplenium serratum* var. *caudatum*, *Asplenium serricula*, *Asplenium sessilifolium*, *Asplenium sessilifolium* var. *guatemalense*, *Asplenium sessilifolium* var. *minus*, *Asplenium sessilifolium* var. *occidentale*, *Asplenium sessilipinnum*, *Asplenium setosum*, *Asplenium shepherdii*, *Asplenium shepherdii* var. *bipinnatum*, *Asplenium shepherdii* var. *flagelliferum*, *Asplenium shikokianum*, *Asplenium simii*, *Asplenium simonsianum*, *Asplenium sintenisii*, *Asplenium skinneri*, *Asplenium skinneri*,
30 *Asplenium sodiroi*, *Asplenium soleiolioides*, *Asplenium solidum* var. *stenophyllum*, *Asplenium solmsii*, *Asplenium* sp.-N.-Halle-2234, *Asplenium spathulinum*, *Asplenium spectabile*, *Asplenium speluncae*, *Asplenium sphaerosporum*, *Asplenium sphenotomum*, *Asplenium spinescens*, *Asplenium splendens*, *Asplenium sprucei*, *Asplenium squamosum* L., *Asplenium standleyi*, *Asplenium stellatum*, *Asplenium stenocarpum*, *Asplenium stoloniferum*, *Asplenium stolonipes*, *Asplenium striatum* L., *Asplenium stuebelianum*, *Asplenium stuhlmannii*, *Asplenium suave*, *Asplenium subalatum*,
35 *Asplenium subcrenatum*, *Asplenium subdigitatum*, *Asplenium subdimidiatum*, *Asplenium subintegrum*, *Asplenium sublaserpitiifolium*, *Asplenium sublongum*, *Asplenium subnudum*, *Asplenium suborbiculare*, *Asplenium subtenuifolium*, *Asplenium subtile*, *Asplenium subtoramanum*, *Asplenium subtrapezoideum*, *Asplenium subvarians*, *Asplenium sulcatum*, *Asplenium sylvaticum*, *Asplenium szechuanense*, *Asplenium taiwanense*, *Asplenium tenerrimum*, *Asplenium tenerum*, *Asplenium tenuicaule*, *Asplenium tenuifolium*, *Asplenium tenuifolium* var. *minor*, *Asplenium tenuifolium* var. *tenuifolium*,
40 *Asplenium tenuissimum*, *Asplenium ternatum*, *Asplenium theciferum*, *Asplenium theciferum* var. *concinnum*, *Asplenium thunbergii*, *Asplenium tianmushanense*, *Asplenium tianshanense*, *Asplenium tibeticum*, *Asplenium tocoraniense*, *Asplenium toramanum*, *Asplenium trapezoideum*, *Asplenium tricholepis*, *Asplenium trichomanes* L., *Asplenium trichomanes* subsp. *inexpectans*, *Asplenium trichomanes* subsp. *quadrivalens*, *Asplenium trichomanes* subsp. *trichomanes*, *Asplenium trichomanes* var. *harovii*, *Asplenium trichomanes* var. *herbaceum*, *Asplenium trichomanes* var. *repens*, *Asplenium trichomanes* var. *viridissimum*, *Asplenium trichomanes-dentatum* L., *Asplenium trigonopterum*, *Asplenium trilobatum*,
45 *Asplenium trilobum*, *Asplenium triphyllum*, *Asplenium triphyllum* var. *compactum*, *Asplenium triphyllum* var. *gracillimum*, *Asplenium triphyllum* var. *herbaceum*, *Asplenium tripteropus*, *Asplenium triquetrum*, *Asplenium truncorum*, *Asplenium tsaratananense*, *Asplenium tucumanense*, *Asplenium tuerckheimii*, *Asplenium tunquiniense*, *Asplenium ulbrichtii*, *Asplenium ultimum*, *Asplenium unilaterale*, *Asplenium unilaterale* var. *decurrens*, *Asplenium unilaterale* var. *udum*, *Asplenium unilaterale* var. *unilaterale*, *Asplenium uniseriale*, *Asplenium uropterum*, *Asplenium vagans*, *Asplenium vareschianum*, *Asplenium variabile* var. *paucijugum*, *Asplenium variabile* var. *variabile*, *Asplenium varians* subsp. *fimbriatum*, *Asplenium varians*, *Asplenium vastum*, *Asplenium venturae*, *Asplenium venulosum*, *Asplenium verapax*, *Asplenium vesiculosum*, *Asplenium vespertinum*, *Asplenium villosum*, *Asplenium virens*, *Asplenium viride*, *Asplenium viridifrons*, *Asplenium virillae*, *Asplenium viviparioides*, *Asplenium viviparum*, *Asplenium viviparum* var. *viviparum*, *Asplenium viviparum* var. *lineatu*, *Asplenium volubile*, *Asplenium vulcanicum*, *Asplenium wacketii*, *Asplenium wagneri*, *Asplenium wallichianum*, *Asplenium warneckei*, *Asplenium wilfordii*, *Asplenium williamsii*, *Asplenium wrightii*, *Asplenium wrightioides*, *Asplenium wuliangshanense*, *Asplenium xianqianense*, *Asplenium xinjiangense*, *Asplenium xinyiense*, *Asplenium yelagagense*, *Asplenium yoshinagae*, *Asplenium yunnanense*, *Asplenium zamiifolium*, *Asplenium zanzibaricum*, *Asple-*

nium biscayneanum, *Asplenium curtissii*, *Asplenium ebenoides*, *Asplenium herb-wagneri*, *Asplenium heteroresiliens*, *Asplenium kenzoii*, *Asplenium plenum*, *Asplenium wangii*, and *Asplenium ×clermontiae*, *Asplenium ×gravesii*.

[0123] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Blechnaceae, Genus *Blechnum* L. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Blechnaceae, Genus *Blechnum* L. selected from but not limited to *Blechnum amabile*, *Blechnum appendiculatum*, *Blechnum articulatum*, *Blechnum australe*, *Blechnum austrobrasilianum*, *Blechnum binervatum*, *Blechnum blechnoides*, *Blechnum brasiliense*, *Blechnum capense*, *Blechnum cartilagineum*, *Blechnum castaneum*, *Blechnum chambersii*, *Blechnum chilense*, *Blechnum colensoi*, *Blechnum contiguum*, *Blechnum cordatum*, *Blechnum coriaceum*, *Blechnum discolor*, *Blechnum doodioides*, *Blechnum durum*, *Blechnum eburneum*, *Blechnum ensiforme*, *Blechnum filiforme*, *Blechnum fluviatile*, *Blechnum fragile*, *Blechnum fraseri*, *Blechnum fullagari*, *Blechnum gibbum*, *Blechnum glandulosum*, *Blechnum gracile*, *Blechnum hancockii*, *Blechnum hastatum*, *Blechnum howeanum*, *Blechnum indicum*, *Blechnum kunthianum*, *Blechnum laevigatum*, *Blechnum loxense*, *Blechnum magellanicum*, *Blechnum membranaceum*, *Blechnum microbasis*, *Blechnum microphyllum*, *Blechnum milnei*, *Blechnum minus*, *Blechnum mochaenum*, *Blechnum montanum*, *Blechnum moorei*, *Blechnum moritzianum*, *Blechnum nigrum*, *Blechnum niponicum*, *Blechnum norfolkianum*, *Blechnum novae-zelandiae*, *Blechnum nudum*, *Blechnum obtusatum*, *Blechnum occidentale*, *Blechnum oceanicum*, *Blechnum orientale*, *Blechnum patersonii*, *Blechnum penna-marina*, *Blechnum polypodioides*, *Blechnum procerum*, *Blechnum punctulatum*, *Blechnum sampaioanum*, *Blechnum schiedeanum*, *Blechnum schomburgkii*, *Blechnum serrulatum*, *Blechnum simillimum*, *Blechnum spicant*, *Blechnum stipitellatum*, *Blechnum tabulare*, *Blechnum triangularifolium*, *Blechnum vieillardii*, *Blechnum vulcanicum*, *Blechnum wattsi*, *Blechnum whelanii*, and *Blechnum wurunuran*.

[0124] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Dryopteridaceae Genus *Acrophorus*, Genus *Acrorumohra*, Genus *Anapausia*, Genus *Arachniodes*, Genus *Bolbitis*, Genus *Ctenitis*, Genus *Cyclodium*, Genus *Cyrtogonellum*, Genus *Cyrtomidictyum*, Genus *Cyrtomium*, Genus *Diacalpe*, Genus *Didymochlaena*, Genus *Dryopsis*, Genus *Dryopteris*, Genus *Elaphoglossum*, Genus *Hypodematium*, Genus *Lastreopsis*, Genus *Leptorumohra*, Genus *Leucostegia*, Genus *Lithostegia*, Genus *Lomagramma*, Genus *Maxonia*, Genus *Megalastrum*, Genus *Olfersia*, Genus *Peranema*, Genus *Phanerophlebia*, Genus *Phanerophlebiopsis*, Genus *Polybotrya*, Genus *Polystichopsis*, Genus *Polystichum*, Genus *Rumohra*, Genus *Sorolepidium*, Genus *Stigmatopteris* or Genus *Teratophyllum*.

[0125] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Dryopteridaceae, Genus *Bolbitis* selected from but not limited to *Bolbitis acrostichoides*, *Bolbitis aliena*, *Bolbitis angustipinna*, *Bolbitis appendiculata*, *Bolbitis auriculata*, *Bolbitis bernoullii*, *Bolbitis bipinnatifida*, *Bolbitis cadieri*, *Bolbitis christensenii*, *Bolbitis confertifolia*, *Bolbitis costata*, *Bolbitis crispatula*, *Bolbitis fluviatilis*, *Bolbitis gaboensis*, *Bolbitis gemmifera*, *Bolbitis hainanensis*, *Bolbitis hastata*, *Bolbitis hekouensis*, *Bolbitis hemiotis*, *Bolbitis heteroclita*, *Bolbitis heudelotii*, *Bolbitis humblotii*, *Bolbitis interlineata*, *Bolbitis latipinna*, *Bolbitis laxireticulata*, *Bolbitis lindigii*, *Bolbitis lonchophora*, *Bolbitis longiflagellata*, *Bolbitis major*, *Bolbitis media*, *Bolbitis nicotianifolia*, *Bolbitis nodiflora*, *Bolbitis novoguineensis*, *Bolbitis oligarchica*, *Bolbitis palustris*, *Bolbitis pandurifolia*, *Bolbitis pergamentacea*, *Bolbitis portoricensis*, *Bolbitis presliana*, *Bolbitis quoyana*, *Bolbitis rawsonii*, *Bolbitis repanda*, *Bolbitis rhizophylla*, *Bolbitis riparia*, *Bolbitis rivularis*, *Bolbitis sagenioides*, *Bolbitis salicina*, *Bolbitis scalpturata*, *Bolbitis scandens*, *Bolbitis semicordata*, *Bolbitis semipinnatifida*, *Bolbitis serrata*, *Bolbitis serratifolia*, *Bolbitis simplex*, *Bolbitis sinensis*, *Bolbitis singaporensis*, *Bolbitis sinuata*, *Bolbitis subcordata*, *Bolbitis subcrenata*, *Bolbitis taylorii*, *Bolbitis tibetica*, *Bolbitis tonkinensis*, *Bolbitis umbrosa*, *Bolbitis vanuaensis*, and *Bolbitis virens*.

[0126] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Lomariopsidaceae, Genus *Nephrolepis*.

[0127] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Lomariopsidaceae, Genus *Nephrolepis* selected from but not limited to *Nephrolepis abrupta*, *Nephrolepis acuminata*, *Nephrolepis acutifolia*, *Nephrolepis arida*, *Nephrolepis arthropteroides*, *Nephrolepis biserrata* var. *auriculata*, *Nephrolepis brownii*, *Nephrolepis celebica*, *Nephrolepis clementis*, *Nephrolepis cordifolia*, *Nephrolepis davalliae*, *Nephrolepis davallioides*, *Nephrolepis dayakorum*, *Nephrolepis delicatula*, *Nephrolepis dicksonioides*, *Nephrolepis duffii*, *Nephrolepis exaltata* ssp. *exaltata* ssp. *Hawaiiensis*, *Nephrolepis falcata*, *Nephrolepis falciformis*, *Nephrolepis glabra*, *Nephrolepis hirsutula*, *Nephrolepis humatoides*, *Nephrolepis iridescens*, *Nephrolepis kurotawae*, *Nephrolepis laurifolia*, *Nephrolepis lauterbachii*, *Nephrolepis lindsayae*, *Nephrolepis multifida*, *Nephrolepis multiflora*, *Nephrolepis niphoboloides*, *Nephrolepis obliterate*, *Nephrolepis paludosa*, *Nephrolepis pectinata*, *Nephrolepis pendula*, *Nephrolepis persicifolia*, *Nephrolepis pickelii*, *Nephrolepis pilosula*, *Nephrolepis pubescens*, *Nephrolepis pumicicola*, *Nephrolepis radicans*, *Nephrolepis rivularis*, *Nephrolepis rosenstockii*, *Nephrolepis saligna*, *Nephrolepis schlechteri*, *Nephrolepis serrate*, *Nephrolepis thomsoni*, *Nephrolepis undulata* var. *aureoglandulosa*, *Nephrolepis x averyi*, *Nephrolepis x copelandii*, and *Nephrolepis x medlerae*.

[0128] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Campyloneurum*, Genus *Drynaria*, Genus *Lepisorus*, Genus *Microgramma*, Genus *Microsorium*,

Genus *Neurodium*, Genus *Niphidium*, Genus *Pecluma* M.G., Genus *Phlebodium*, Genus *Phymatosorus*, Genus *Platyserium*, Genus *Pleopeltis*, Genus *Polypodium* L.

[0129] In some embodiments the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Polypodium* L.

5 [0130] In some embodiments the PtlP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Polypodium* L. selected from but not limited to *Polypodium absidatum*, *Polypodium acutifolium*, *Polypodium adiantiforme*, *Polypodium aequale*, *Polypodium affine*, *Polypodium albidopaleatum*, *Polypodium alcorne*, *Polypodium alfarii*, *Polypodium alfredii*, *Polypodium alfredii* var. *curtii*, *Polypodium allosuroides*, *Polypodium alsophilicola*, *Polypodium amamanum*, *Polypodium amoenum*, *Polypodium amorphum*, *Polypodium anetioides*, *Polypodium anfractu-
10* *osum*, *Polypodium anguinum*, *Polypodium angustifolium* f. *remotifolia*, *Polypodium angustifolium* var. *amphostenon*, *Polypodium angustifolium* var. *heterolepis*, *Polypodium angustifolium* var. *monstrosa*, *Polypodium angustipaleatum*, *Polypodium angustissimum*, *Polypodium anisomeron* var. *pectinatum*, *Polypodium antioquianum*, *Polypodium aoristis-
15* *sorum*, *Polypodium apagolepis*, *Polypodium apicidens*, *Polypodium apiculatum*, *Polypodium apoense*, *Polypodium ap-
alachianum*, *Polypodium appressum*, *Polypodium arenarium*, *Polypodium argentinum*, *Polypodium argutum*, *Polypo-
20* *dium armatum*, *Polypodium aromaticum*, *Polypodium aspersum*, *Polypodium assurgens*, *Polypodium atrum*, *Polypodium auriculatum*, *Polypodium balaonense*, *Polypodium balliviani*, *Polypodium bamleri*, *Polypodium bangii*, *Polypodium bartlettii*, *Polypodium basale*, *Polypodium bernoullii*, *Polypodium biauratum*, *Polypodium bifrons*, *Polypodium blepharodes*, *Polypodium bolivari*, *Polypodium bolivianum*, *Polypodium bolobense*, *Polypodium bombycinum*, *Polypodium bombycinum* var. *insularum*, *Polypodium bradeorum*, *Polypodium bryophilum*, *Polypodium bryopodium*, *Polypodium buchtienii*, *Polypodium buesii*, *Polypodium bulbotrichum*, *Polypodium caceresii*, *Polypodium californicum* f. *brauscombi*, *Polypo-
25* *dium californicum* f. *parsonsia*, *Polypodium californicum*, *Polypodium calophlebium*, *Polypodium calvum*, *Polypodium camptophyllarium* var. *abbreviatum*, *Polypodium capitellatum*, *Polypodium carpintera*, *Polypodium chachapoyense*, *Polypodium chartaceum*, *Polypodium chimantense*, *Polypodium chiricanum*, *Polypodium choquetangense*, *Polypodium christensenii*, *Polypodium christii*, *Polypodium chrysotrichum*, *Polypodium ciliolepis*, *Polypodium cinerascens*, *Polypo-
30* *dium collinsii*, *Polypodium colysoides*, *Polypodium confluens*, *Polypodium conforme*, *Polypodium confusum*, *Polypodium congregatifolium*, *Polypodium connellii*, *Polypodium consimile* var. *bourgaeum*, *Polypodium consimile* var. *minor*, *Polypodium conterminans*, *Polypodium contiguum*, *Polypodium cookii*, *Polypodium coriaceum*, *Polypodium coronans*, *Polypodium costaricense*, *Polypodium costatum*, *Polypodium crassifolium* f. *angustissimum*, *Polypodium crassifolium* var. *longipes*, *Polypodium crassulum*, *Polypodium craterisorum*, *Polypodium cryptum*, *Polypodium crystalloneuron*, *Polypo-
35* *dium cucullatum* var. *planum*, *Polypodium cuencanum*, *Polypodium cumingianum*, *Polypodium cupreolepis*, *Polypo-
dium curranii*, *Polypodium curvans*, *Polypodium cyathicola*, *Polypodium cyathisorum*, *Polypodium cyclocolpon*, *Polypo-
dium daguense*, *Polypodium damunense*, *Polypodium dareiformioides*, *Polypodium dasypleura*, *Polypodium decipiens*, *Polypodium decorum*, *Polypodium delicatulum*, *Polypodium deltoideum*, *Polypodium demeraranum*, *Polypodium den-
40* *ticulatum*, *Polypodium diaphanum*, *Polypodium dilatatum*, *Polypodium dispersum*, *Polypodium dissectum*, *Polypodium dissimulans*, *Polypodium dolichosorum*, *Polypodium dolorense*, *Polypodium donnell-smithii*, *Polypodium drymoglos-
45* *soides*, *Polypodium ebeninum*, *Polypodium eggersii*, *Polypodium elmeri*, *Polypodium elongatum*, *Polypodium enteroso-
roides*, *Polypodium erubescens*, *Polypodium erythrolepis*, *Polypodium erythrotrichum*, *Polypodium eurybasis*, *Polypo-
dium eurybasis* var. *villosum*, *Polypodium exornans*, *Polypodium falcoideum*, *Polypodium fallacissimum*, *Polypodium farinosum*, *Polypodium faucium*, *Polypodium feei*, *Polypodium ferrugineum*, *Polypodium feuillei*, *Polypodium firmulum*, *Polypodium firmum*, *Polypodium flaccidum*, *Polypodium flagellare*, *Polypodium flexuosum*, *Polypodium flexuosum* var. *ekmanii*, *Polypodium forbesii*, *Polypodium formosanum*, *Polypodium fraxinifolium* subsp. *articulatum*, *Polypodium frax-
50* *inifolium* subsp. *luridum*, *Polypodium fructuosum*, *Polypodium fucoides*, *Polypodium fulvescens*, *Polypodium galeottii*, *Polypodium glaucum*, *Polypodium glycyrrhiza*, *Polypodium gracillimum*, *Polypodium gramineum*, *Polypodium grandifolium*, *Polypodium gratum*, *Polypodium graveolens*, *Polypodium griseo-nigrum*, *Polypodium griseum*, *Polypodium gut-
55* *tatum*, *Polypodium haalilioanum*, *Polypodium hammatisorum*, *Polypodium hancockii*, *Polypodium haplophlebicum*, *Polypo-
dium harrisii*, *Polypodium hastatum* var. *simplex*, *Polypodium hawaiiense*, *Polypodium heanophyllum*, *Polypodium helleri*, *Polypodium hemionitidium*, *Polypodium henryi*, *Polypodium herzogii*, *Polypodium hesperium*, *Polypodium hessii*, *Polypodium hombersleyi*, *Polypodium hostmannii*, *Polypodium humile*, *Polypodium hyalinum*, *Polypodium iboense*, *Polypo-
dium induens* var. *subdentatum*, *Polypodium insidiosum*, *Polypodium insigne*, *Polypodium intermedium* subsp. *ma-
60* *safueranum* var. *obtusesserratum*, *Polypodium intramarginale*, *Polypodium involutum*, *Polypodium itatiayense*, *Polypo-
dium javanicum*, *Polypodium juglandifolium*, *Polypodium kaniense*, *Polypodium knowltoniorum*, *Polypodium kyimbilense*, *Polypodium l'herminieri* var. *costaricense*, *Polypodium lachniferum* f. *incurvata*, *Polypodium lachniferum* var. *glabres-
65* *cens*, *Polypodium lachnopus*, *Polypodium lanceolatum* var. *complanatum*, *Polypodium lanceolatum* var. *trichophorum*, *Polypodium latevagans*, *Polypodium laxifrons*, *Polypodium laxifrons* var. *lividum*, *Polypodium lehmannianum*, *Polypo-
dium leiorhizum*, *Polypodium leptopodon*, *Polypodium leuconeuron* var. *angustifolia*, *Polypodium leuconeuron* var. *lat-
70* *ifolium*, *Polypodium leucosticta*, *Polypodium limulum*, *Polypodium lindigii*, *Polypodium lineatum*, *Polypodium lomarioides*, *Polypodium longifrons*, *Polypodium loretense*, *Polypodium loriceum* var. *umbraticum*, *Polypodium loriforme*, *Polypodium loxogramme* f. *gigas*, *Polypodium ludens*, *Polypodium luzonicum*, *Polypodium lycopodioides* f. *obtusum*, *Polypodium*

lycopodioides L., *Polypodium macrolepis*, *Polypodium macrophyllum*, *Polypodium macrosorum*, *Polypodium macrosphaerum*, *Polypodium maculosum*, *Polypodium madreense*, *Polypodium manmeiense*, *Polypodium margaritifera*, *Polypodium maritimum*, *Polypodium martensii*, *Polypodium majoris*, *Polypodium megalolepis*, *Polypodium melanotrichum*, *Polypodium menisciifolium* var. *pubescens*, *Polypodium meniscioides*, *Polypodium merrillii*, *Polypodium mettenii*,
5 *Polypodium mexiae*, *Polypodium microsorum*, *Polypodium militare*, *Polypodium minimum*, *Polypodium minusculum*, *Polypodium mixtum*, *Polypodium mollendense*, *Polypodium mollissimum*, *Polypodium moniliforme* var. *minus*, *Polypodium monoides*, *Polypodium monticola*, *Polypodium montigenum*, *Polypodium moritzianum*, *Polypodium moultonii*, *Polypodium multicaudatum*, *Polypodium multilineatum*, *Polypodium multisorum*, *Polypodium munchii*, *Polypodium muscoides*, *Polypodium myriolepis*, *Polypodium myriophyllum*, *Polypodium myriotrichum*, *Polypodium nematorhizon*, *Polypodium nemorale*, *Polypodium nesioticum*, *Polypodium nigrescentium*, *Polypodium nigripes*, *Polypodium nigrocinctum*,
10 *Polypodium nimbatum*, *Polypodium nitidissimum*, *Polypodium nitidissimum* var. *latior*, *Polypodium nubrigenum*, *Polypodium oligolepis*, *Polypodium oligosorum*, *Polypodium oligosorum*, *Polypodium olivaceum*, *Polypodium olivaceum* var. *elatum*, *Polypodium oodes*, *Polypodium oosphaerum*, *Polypodium oreophilum*, *Polypodium ornatissimum*, *Polypodium ornatum*, *Polypodium ovatum*, *Polypodium oxylobum*, *Polypodium oxypholis*, *Polypodium pakkaense*, *Polypodium pallidum*, *Polypodium palmatopedatum*, *Polypodium palmeri*, *Polypodium panamense*, *Polypodium parvum*, *Polypodium patagonicum*, *Polypodium paucisorum*, *Polypodium pavonianum*, *Polypodium pectinatum* var. *caliense*, *Polypodium pectinatum* var. *hispidum*, *Polypodium pellucidum*, *Polypodium pendulum* var. *boliviense*, *Polypodium percrassum*, *Polypodium perpusillum*, *Polypodium peruvianum* var. *subgibbosum*, *Polypodium phyllitidis* var. *elongatum*, *Polypodium pichinchense*, *Polypodium pilosissimum*, *Polypodium pilosissimum* var. *glabriusculum*, *Polypodium pilosissimum* var. *tunguraguensis*, *Polypodium pityrolepis*, *Polypodium platyphyllum*, *Polypodium playfairii*, *Polypodium plebeium* var. *cooperi*,
20 *Polypodium plectolepidioides*, *Polypodium pleolepis*, *Polypodium plesiosorum* var. *i*, *Polypodium podobasis*, *Polypodium podocarpum*, *Polypodium poloense*, *Polypodium polydatylon*, *Polypodium polypodioides* var. *aciculare*, *Polypodium polypodioides* var. *michauxianum*, *Polypodium praetermissum*, *Polypodium preslianum* var. *immersum*, *Polypodium procerum*, *Polypodium procerum*, *Polypodium productum*, *Polypodium productum*, *Polypodium prolongilobum*, *Polypodium propinguum*, *Polypodium proteus*, *Polypodium pruinaum*, *Polypodium pseudocapillare*, *Polypodium pseudofraternum*, *Polypodium pseudonutans*, *Polypodium pseudoserratum*, *Polypodium pulcherrimum*, *Polypodium pulogense*, *Polypodium pungens*, *Polypodium purpusii*, *Polypodium radicale*, *Polypodium randallii*, *Polypodium ratiborii*, *Polypodium reclinatum*, *Polypodium recreense*, *Polypodium repens* var. *abruptum*, *Polypodium revolvens*, *Polypodium rhachipterygium*, *Polypodium rhomboideum*, *Polypodium rigens*, *Polypodium robustum*, *Polypodium roraimense*, *Polypodium roraimense*, *Polypodium rosei*, *Polypodium rosenstockii*, *Polypodium rubidum*, *Polypodium rudimentum*, *Polypodium rusbyi*, *Polypodium sablanianum*, *Polypodium sarmentosum*, *Polypodium saxicola*, *Polypodium schenckii*, *Polypodium schlechteri*, *Polypodium scolopendria*, *Polypodium scolopendria*, *Polypodium scolopendrium*, *Polypodium scouleri*, *Polypodium scutulatum*, *Polypodium segregatum*, *Polypodium semihirsutum*, *Polypodium semihirsutum* var. *fuscetosum*, *Polypodium senile* var. *minor*, *Polypodium sericeolanatum*, *Polypodium serraeforme*, *Polypodium serricula*, *Polypodium sesquipedala*, *Polypodium sessilifolium*, *Polypodium setosum* var. *calvum*, *Polypodium setulosum*, *Polypodium shaferi*, *Polypodium sibomense*, *Polypodium siccum*, *Polypodium simacense*, *Polypodium simulans*, *Polypodium singeri*, *Polypodium sinicum*, *Polypodium sintensisii*, *Polypodium skutchii*, *Polypodium sloanei*, *Polypodium sodiroi*, *Polypodium sordidulum*, *Polypodium sordidum*, *Polypodium sphaeropteroides*, *Polypodium sphenodes*, *Polypodium sprucei*, *Polypodium sprucei* var. *furcativenosa*, *Polypodium steirolepis*, *Polypodium stenobasis*, *Polypodium stenolepis*, *Polypodium stenopterum*, *Polypodium subcapillare*, *Polypodium subflabelliforme*, *Polypodium subhemionitidium*, *Polypodium subinaequale*, *Polypodium subintegrum*, *Polypodium subspathulatum*, *Polypodium subtile*, *Polypodium subvestitum*, *Polypodium subviride*, *Polypodium superficiale* var. *attenuatum*, *Polypodium superficiale* var. *chinensis*, *Polypodium sursumcurrens*, *Polypodium tablazianum*, *Polypodium taenifolium*, *Polypodium tamandarei*, *Polypodium tatei*, *Polypodium tenuiculum* var. *acrosora*, *Polypodium tenuiculum* var. *brasiliense*, *Polypodium tenuilore*, *Polypodium tenuinerve*, *Polypodium tepuiense*, *Polypodium teresae*, *Polypodium tetragonum* var. *incompletum*, *Polypodium thysanolepis* var. *bipinnatifidum*, *Polypodium thyssanolepis*, var. *thyssanolepis*, *Polypodium thyssanolepsi*, *Polypodium tobagense*, *Polypodium trichophyllum*, *Polypodium tridactylum*, *Polypodium tridentatum*, *Polypodium trifurcatum* var. *brevipes*, *Polypodium triglossum*, *Polypodium truncatulum*, *Polypodium truncicola* var. *major*, *Polypodium truncicola* var. *minor*, *Polypodium tuberosum*, *Polypodium tunguraguae*, *Polypodium turquinum*, *Polypodium turrialbae*, *Polypodium ursipes*, *Polypodium vagans*, *Polypodium valdealatum*, *Polypodium versteegii*, *Polypodium villagrani*, *Polypodium virginianum* f. *cambroideum*, *Polypodium virginianum* f. *periferens*, *Polypodium vittarioides*, *Polypodium vulgare*, *Polypodium vulgare* L., *Polypodium vulgare* subsp. *oreophilum*, *Polypodium vulgare* var. *acuminatum*, *Polypodium vulpinum*, *Polypodium williamsii*, *Polypodium wobbenense*, *Polypodium x fallacissimum-guttatum*, *Polypodium xantholepis*, *Polypodium xiphopteris*, *Polypodium yarumalense*, *Polypodium yungense*, and *Polypodium zosteriforme*.
55

[0131] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platycterium*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platycterium* selected from but not limited to *Platycterium alcorni*, *Platycterium andinum*, *Platycterium angolense*, *Platycterium bifurcatum*, *Platycterium coronarium*, *Platycterium elephan-*

totis, Platycerium ellisii, Platycerium grande, Platycerium hillii, Platycerium holttumii, Platycerium madagascariense, Platycerium quadridichotomum, Platycerium ridleyi, Platycerium sp. ES-2011, Platycerium stemaria, Platycerium superbum, Platycerium veitchii, Platycerium wallichii, Platycerium wandae, Platycerium wilhelminae-reginae, and Platycerium willinckii.

[0132] In some embodiments the PtIP-50 polypeptide is derived from a species in the Division *Lycophyta*.

[0133] In some embodiments the PtIP-50 polypeptide is derived from a species in the Class *Isoetopsida* or Class *Lycopodiopsida*.

[0134] In some embodiments the PtIP-50 polypeptide is derived from a species in the Class *Isoetopsida* Order *Selaginales*. In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Isoetopsida*, Order *Selaginales*, Family *Selaginellaceae*. In some embodiments the PtIP-50 polypeptide is derived from a species in the Genus *Selaginella*. In some embodiments the PtIP-50 polypeptide is derived from a *Selaginella* species selected from but not limited to *Selaginella acanthonota, Selaginella apoda, Selaginella arbuscula, Selaginella arenicola, Selaginella arizonica, Selaginella armata, Selaginella asprella, Selaginella biformis, Selaginella bigelovii, Selaginella braunii, Selaginella cinerascens, Selaginella cordifolia, Selaginella deflexa, Selaginella delicatula, Selaginella densa, Selaginella douglasii, Selaginella eatonii, Selaginella eclipses, Selaginella eremophila, Selaginella erythropus, Selaginella flabellata, Selaginella hansenii, Selaginella heterodonta, Selaginella kraussiana, Selaginella krugii, Selaginella laxifolia, Selaginella lepidophylla, Selaginella leucobryoides, Selaginella ludoviciana, Selaginella mutica, Selaginella oregana, Selaginella ovifolia, Selaginella pallascens, Selaginella peruviana, Selaginella pilifera, Selaginella plana, Selaginella plumosa, Selaginella pulcherrima, Selaginella rupestris, Selaginella rupincola, Selaginella scopulorum, Selaginella selaginoides, Selaginella sibirica, Selaginella standleyi, Selaginella stellata, Selaginella subcaulescens, Selaginella substipitata, Selaginella tenella, Selaginella tortipila, Selaginella uliginosa, Selaginella umbrosa, Selaginella uncinata, Selaginella underwoodii, Selaginella utahensis, Selaginella victoriae, Selaginella viridissima, Selaginella wallacei, Selaginella watsonii, Selaginella weatherbiana, Selaginella willdenowii, Selaginella wrightii and Selaginella X neomexicana.*

[0135] In some embodiments the PtIP-50 polypeptide is derived from a species in the Class *Lycopodiopsida*, Order *Lycopodiales*.

[0136] In some embodiments the PtIP-50 polypeptide is derived from a fern species in the Class *Lycopodiopsida*, Order *Lycopodiales* Family *Lycopodiaceae* or Family *Huperziaceae*.

[0137] In some embodiments the PtIP-50 polypeptide is derived from a species in the Genus *Austrolycopodium, Dendrolycopodium, Diphasiastrum, Diphasium, Huperzia, Lateristachys, Lycopodiastrum, Lycopodiella, Lycopodium, Palhinhaea, Pseudodiphasium, Pseudolycopodiella, Pseudolycopodium* or *Spinulum*.

[0138] In some embodiments the PtIP-50 polypeptide is derived from a species in the Genus *Lycopodium*.

[0139] In some embodiments the PtIP-50 polypeptide is derived from a *Lycopodium* species selected from but not limited to *Lycopodium alpinum L., Lycopodium annotinum L., Lycopodium clavatum L., Lycopodium complanatum L., Lycopodium dendroideum Michx., Lycopodium digitatum, Lycopodium xhabereri, Lycopodium hickeyi, Lycopodium xissleri, Lycopodium lagopus, Lycopodium obscurum L., Lycopodium phlegmaria L., Lycopodium sabinifolium, Lycopodium sitchense, Lycopodium tristachyum, Lycopodium venustulum, Lycopodium venustulum var. venustulum, Lycopodium venustulum var. verticale, Lycopodium volubile and Lycopodium xzeilleri.*

[0140] In some embodiments the PtIP-50 polypeptide is derived from a species in the Genus *Huperzia*. In some embodiments the PtIP-50 polypeptide is derived from a species selected from but not limited to *Huperzia appressa, Huperzia arctica, Huperzia attenuata, Huperzia australiana, Huperzia balansae, Huperzia billardierei, Huperzia brassii, Huperzia campiana, Huperzia capellae, Huperzia carinata, Huperzia cf. carinata ARF000603, Huperzia cf. nummulariifolia ARF001140, Huperzia cf. phlegmaria ARF000717, Huperzia cf. phlegmaria ARF000771, Huperzia cf. phlegmaria ARF000785, Huperzia cf. phlegmaria ARF001007, Huperzia cf. phlegmaria ARF002568, Huperzia cf. phlegmaria ARF002703, Huperzia cf. phlegmaria Wikstrom 1998, Huperzia chinensis, Huperzia compacta, Huperzia crassa, Huperzia crispata, Huperzia cryptomeriana, Huperzia cumingii, Huperzia dacrydioides, Huperzia dalhousieana, Huperzia dichotoma, Huperzia emeiensis, Huperzia ericifolia, Huperzia eversa, Huperzia fargesii, Huperzia fordii, Huperzia funiformis, Huperzia goebellii, Huperzia haleakalae, Huperzia hamiltonii, Huperzia heteroclita, Huperzia hippuridea, Huperzia hippuris, Huperzia holstii, Huperzia horizontalis, Huperzia hunanensis, Huperzia hystrix, Huperzia lindenii, Huperzia linifolia, Huperzia lockyeri, Huperzia lucidula, Huperzia mingcheensis, Huperzia miyoshiana, Huperzia nanchuanensis, Huperzia nummulariifolia, Huperzia obtusifolia, Huperzia ophioglossoides, Huperzia petiolata, Huperzia phlegmaria, Huperzia phlegmarioides, Huperzia phyllantha, Huperzia piniifolia, Huperzia polydactyla, Huperzia prolifera, Huperzia reflexa, Huperzia rosenstockiana, Huperzia rufescens, Huperzia salvinoides, Huperzia sarmentosa, Huperzia selago, Huperzia serrata, Huperzia sieboldii, Huperzia somae, Huperzia squarrosa, Huperzia subulata, Huperzia sutchueniana, Huperzia tauri, Huperzia taxifolia, Huperzia tenuis, Huperzia tetragona, Huperzia tetrasticha, Huperzia unguiculata, Huperzia varia, Huperzia verticillata and Huperzia wilsonii.*

Phylogenetic, sequence motif, and structural analyses for insecticidal protein families

[0141] The sequence and structure analysis method employed is composed of four components: phylogenetic tree construction, protein sequence motifs finding, secondary structure prediction, and alignment of protein sequences and secondary structures. Details about each component are illustrated below.

1) Phylogenetic tree construction

[0142] The phylogenetic analysis was performed using the software MEGA5. Protein sequences were subjected to ClustalW version 2 analysis (Larkin M.A et al (2007) *Bioinformatics* 23(21): 2947-2948) for multiple sequence alignment. The evolutionary history was then inferred by the Maximum Likelihood method based on the JTT matrix-based model. The tree with the highest log likelihood was obtained, exported in Newick format, and further processed to extract the sequence IDs in the same order as they appeared in the tree. A few clades representing sub-families were manually identified for each insecticidal protein family.

2) Protein sequence motifs finding

[0143] Protein sequences were re-ordered according to the phylogenetic tree built previously, and fed to the motif analysis tool MEME (Multiple EM for motif Elicitation) (Bailey T.L., and Elkan C., *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*, pp. 28-36, AAAI Press, Menlo Park, California, 1994.) for identification of key sequence motifs. MEME was setup as follows: Minimum number of sites 2, Minimum motif width 5, and Maximum number of motifs 50. Sequence motifs unique to each sub-family were identified by visual observation. The distribution of motifs across the entire gene family could be visualized in HTML webpage. The motifs are numbered relative to the ranking of the E-value for each motif. The amino acid sequence motifs identified for each of the PtIP-50 polypeptides and the residue ranges defining the motifs are shown in Table 2. An amino acid sequence motif not identified in a particular PtIP-50 polypeptide is indicated in Table 2 as "n. i.". The amino acid sequence motifs identified for each of the PtIP-65 polypeptides and the residue ranges defining the motifs are shown in Table 3. An amino acid sequence motif not identified in a particular PtIP-65 polypeptide is indicated in Table 3 as "n. i.". Figure 16a - 16u shows an alignment of the PtIP-50 polypeptides PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, PtIP-50Bd, PtIP-50Fa, PtIP-50Fb, PtIP-50Fd, PtIP-50Fe, PtIP-50Ff, PtIP-50Fg, PtIP-50Fh, PtIP-50Fi, PtIP-50Fj, PtIP-50Fk, PtIP-50Fl, PtIP-50Fm, PtIP-50Fn, PtIP-50Fo, PtIP-50Fp, PtIP-50Fq, PtIP-50Fr, PtIP-50Fs, PtIP-50Ft, PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, and PtIP-50Gd, and the location, relative to PtIP-50Fb, of the amino acid sequence motifs present in PtIP-50Fb. Figure 17a - 17k shows an alignment of the amino acid sequences of PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, PtIP-65Ca, PtIP-65Fa, PtIP-65Fb, PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Gd, PtIP-65Ge, PtIP-65Ha, PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, PtIP-65Hf, PtIP-65Hg, PtIP-65Hh, PtIP-65Hj, and PtIP-65Hk; and the location, relative to PtIP-65Gc, of the amino acid sequence motifs present in PtIP-65Gc.

3) Secondary structure prediction

[0144] PSIPRED, top ranked secondary structure prediction method (Jones DT. (1999) *J. Mol. Biol.* 292: 195-202), was installed in local Linux server, and used for protein secondary structure prediction. The tool provides accurate structure prediction using two feed-forward neural networks based on the PSI-BLAST output. The PSI-BLAST database was created by removing low-complexity, transmembrane, and coiled-coil regions in Uniref100. The PSIPRED results contain the PtIP- secondary structures (Alpha helix: H, Beta strand: E, and Coil: C) and the corresponding confidence scores for each amino acid in a given protein sequence. Figure 16 shows the PtIP-50 polypeptide amino acid sequence alignments and the conserved secondary structural regions. Figure 17 shows the PtIP-65 polypeptide amino acid sequence alignments and conserved secondary structural regions.

4) Alignment of protein sequences and secondary structures

[0145] A customized script was developed to generate gapped secondary structure alignment according to the multiple protein sequence alignment from step 1 for all proteins. All aligned protein sequences and structures were concatenated into a single FASTA file, and then imported into MEGA for visualization and identification of conserved structures. The file was also edited in GeneDoc to produce acceptable sequence format for patent offices. In some embodiments a PtIP-50 polypeptide has a calculated molecular weight of between about 70kD and about 120kD, between about 75kD and about 110kD, between about 80kD and about 105kD, or between about 85kD and about 105kD.

[0146] In some embodiments the PtIP-50 polypeptide has a modified physical property. As used herein, the term "physical property" refers to any parameter suitable for describing the physical-chemical characteristics of a protein. As

used herein, "physical property of interest" and "property of interest" are used interchangeably to refer to physical properties of proteins that are being investigated and/or modified. Examples of physical properties include, but are not limited to net surface charge and charge distribution on the protein surface, net hydrophobicity and hydrophobic residue distribution on the protein surface, surface charge density, surface hydrophobicity density, total count of surface ionizable groups, surface tension, protein size and its distribution in solution, melting temperature, heat capacity, and second virial coefficient. Examples of physical properties also include, but are not limited to solubility, folding, stability, and digestibility. In some embodiments the PtIP-50 polypeptide has increased digestibility of proteolytic fragments in an insect gut. Models for digestion by simulated simulated gastric fluids are known to one skilled in the art (Fuchs, R.L. and J.D. Astwood. Food Technology 50: 83-88, 1996; Astwood, J.D., et al Nature Biotechnology 14: 1269-1273, 1996; Fu TJ et al J. Agric Food Chem. 50: 7154-7160, 2002).

PtIP-65 polypeptides

[0147] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Division *Pteridophyta*. The phylogeny of ferns as used herein is based on the classification for extant ferns by A. R. Smith et al, TAXON, 55:705-731 (2006). The consensus phylogeny based on the classification by A. R. Smith is shown in Figure 1. Additional information on the phylogeny of ferns can be found at mobot.org/MOBOT/research/APweb/ (which can be accessed using the "www" prefix) and Schuettpeiz E. and Pryer K. M., TAXON 56: 1037-1050 (2007) based on three plastid genes. Additional fern and other primitive plant species can be found at homepages.caverock.net.nz/~bj/fern/list.htm (which can be accessed using the http:// prefix).

[0148] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Psilotales*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales*, Family *Psilotaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Psilotopsida*, Order *Ophioglossales* Family *Ophioglossaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Genus *Ophioglossum* L., *Botrychium*, *Botrypus*, *Helminthostachys*, *Ophioderma*, *Cheiroglossa*, *Sceptridium* or *Mankyua*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the *Ophioglossum* L. Genus is selected from but not limited to *Ophioglossum californicum*, *Ophioglossum coriaceum*, *Ophioglossum costatum*, *Ophioglossum crotalophoroides*, *Ophioglossum engelmannii*, *Ophioglossum falcatum*, *Ophioglossum gomezianum*, *Ophioglossum gramineum*, *Ophioglossum kawamurae*, *Ophioglossum lusitanicum*, *Ophioglossum namegatae*, *Ophioglossum nudicaule*, *Ophioglossum palmatum*, *Ophioglossum parvum*, *Ophioglossum pedunculolum*, *Ophioglossum pendulum*, *Ophioglossum petiolatum*, *Ophioglossum pusillum*, *Ophioglossum reticulatum*, *Ophioglossum richardsiae*, *Ophioglossum thermale*, and *Ophioglossum vulgatum*.

[0149] In some embodiments the PtIP-65 polypeptide is derived from a species in the Class *Polypodiopsida/Pteridopsida*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Osmundales* (royal ferns); Family *Osmundaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Hymenophyllales* (filmy ferns and bristle ferns); Family *Hymenophyllaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Gleicheniales*; Family *Gleicheniaceae*, Family *Dipteridaceae* or Family *Matoniaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Schizaeales; Family *Lygodiaceae*, Family *Anemiaceae* or Family *Schizaeaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Salviniales*; Family *Marsileaceae* or Family *Salviniaceae*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Cyatheaales*; Family *Thyrsopteridaceae*, Family *Loxosomataceae*, Family *Culcitaceae*, Family *Plagiogyriaceae*, Family *Cibotiaceae*, Family *Cyatheaceae*, Family *Dicksoniaceae* or Family *Metaxyaceae*.

[0150] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Polypodiales*; Family *Lindsaeaceae*, Family *Saccolomataceae*, Family *Cystodiaceae*, Family *Dennstaedtiaceae*, Family *Pteridaceae*, Family *Aspleniaceae*, Family *Thelypteridaceae*, Family *Woodsiaceae*, Family *Onocleaceae*, Family *Blechnaceae*, Family *Dryopteridaceae*, Family *Lomariopsidaceae*, Family *Tectariaceae*, Family *Oleandraceae*, Family *Davalliaceae* or Family *Polypodiaceae*.

[0151] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order *Polypodiales*, Family *Pteridaceae*, Genus *Adiantaceae* selected from but not limited to *Adiantum aethiopicum*, *Adiantum aleuticum*, *Adiantum bonatianum*, *Adiantum cajennense*, *Adiantum capillus-junonis*, *Adiantum capillus-veneris*, *Adiantum caudatum*, *Adiantum chienii*, *Adiantum chilense*, *Adiantum cuneatum*, *Adiantum cunninghamii*, *Adiantum davidii*, *Adiantum diaphanum*, *Adiantum edentulum*, *Adiantum edgeworthii*, *Adiantum excisum*, *Adiantum fengianum*, *Adiantum fimbriatum*, *Adiantum flabellulatum*, *Adiantum formosanum*, *Adiantum formosum*, *Adiantum fulvum*, *Adiantum gravesii*, *Adiantum hispidulum*, *Adiantum induratum*, *Adiantum jordanii*, *Adiantum juxtapositum*, *Adiantum latifolium*, *Adiantum leveillei*, *Adiantum lianxianense*, *Adiantum malesianum*, *Adiantum mariesii*, *Adiantum monochlamys*, *Adiantum myriosorum*, *Adiantum obliquum*,

Adiantum ogasawarensense, *Adiantum pedatum*, *Adiantum pentadactylon*, *Adiantum peruvianum*, *Adiantum philippense*, *Adiantum princeps*, *Adiantum pubescens*, *Adiantum raddianum*, *Adiantum reniforme*, *Adiantum roborowskii*, *Adiantum serratodentatum*, *Adiantum sinicum*, *Adiantum soboliferum*, *Adiantum subcordatum*, *Adiantum tenerum*, *Adiantum terminatum*, *Adiantum tetraphyllum*, *Adiantum venustum*, *Adiantum viridescens*, and *Adiantum viridimontanum*.

5 **[0152]** In some embodiments the PtlP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Aspleniaceae, Genus *Asplenium* L. In some embodiments the PtlP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Aspleniaceae, Genus *Asplenium* L selected from but not limited to *Asplenium abbreviatum*, *Asplenium abrotanoides*, *Asplenium abscissum* var. *subaequilaterale*, *Asplenium abscissum*, *Asplenium achilleifolium*,
 10 *Asplenium acuminatum*, *Asplenium adiantifrons*, *Asplenium adiantoides*, *Asplenium adiantoides* var. *squamulosum*, *Asplenium adiantum-nigrum* L., *Asplenium adiantum-nigrum* var. *adiantum-nigrum*, *Asplenium adiantum-nigrum* var. *yuanum*, *Asplenium adnatum*, *Asplenium aethiopicum*, *Asplenium affine*, *Asplenium affine* var. *affine*, *Asplenium affine* var. *gilpinae*, *Asplenium affine* var. *mettenii*, *Asplenium affine* var. *pecten*, *Asplenium africanum*, *Asplenium afzelii*, *Asplenium aitchisonii*, *Asplenium alatulum*, *Asplenium alatum*, *Asplenium alfredii*, *Asplenium altajense*, *Asplenium amabile*, *Asplenium ambohitantelense*, *Asplenium anceps* var. *proliferum*, *Asplenium andapense*, *Asplenium andersonii*,
 15 *Asplenium angustatum*, *Asplenium angustum*, *Asplenium anisophyllum*, *Asplenium annetii*, *Asplenium antiquum*, *Asplenium antrophyoides*, *Asplenium apertum*, *Asplenium apogamum*, *Asplenium aquaticum*, *Asplenium arboreum*, *Asplenium arcanum*, *Asplenium arcuatum*, *Asplenium argentinum*, *Asplenium argutum*, *Asplenium aspidiiforme*, *Asplenium aspidioides*, *Asplenium asterolepis*, *Asplenium auricularium* var. *acutidens*, *Asplenium auricularium* var. *subintegerrimum*, *Asplenium auriculatum*, *Asplenium auriculatum* var. *aequilaterale*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *diversifolium*, *Asplenium auritum* fo. *nana*, *Asplenium auritum*, *Asplenium auritum* var. *auriculatum*, *Asplenium auritum* var. *auritum*, *Asplenium auritum* var. *bipinnatifidum*, *Asplenium auritum* var. *bipinnatisectum*, *Asplenium auritum* var. *davallioides*, *Asplenium auritum* var. *macilentum*, *Asplenium auritum* var. *rigidum*, *Asplenium auritum* var. *subsimpler*, *Asplenium austrochinense*, *Asplenium ayopayense*, *Asplenium badinii*, *Asplenium balense*, *Asplenium ballivianii*, *Asplenium bangii*, *Asplenium bangii*, *Asplenium barbaense*, *Asplenium barclayanum*, *Asplenium barkamense*, *Asplenium barteri*, *Asplenium basispicum*, *Asplenium bicrenatum*, *Asplenium bifrons*, *Asplenium bipartitum*, *Asplenium blastophorum*, *Asplenium blepharodes*, *Asplenium blepharophorum*, *Asplenium boiteaui*, *Asplenium bolivianum*, *Asplenium boltonii*, *Asplenium borealichinense*, *Asplenium bradei*, *Asplenium bradeorum*, *Asplenium bradleyi*, *Asplenium brausei*, *Asplenium breedlovei*, *Asplenium buettneri*, *Asplenium buettneri* var. *hildebrandtii*, *Asplenium bulbiferum*, *Asplenium bullatum* var. *bullatum*, *Asplenium bullatum* var. *shikokianum*, *Asplenium bullatum*, *Asplenium cancellatum*, *Asplenium capillipes*, *Asplenium cardiophyllum* (Hance), *Asplenium caripense*, *Asplenium carvalhoanum*, *Asplenium castaneoviride*, *Asplenium castaneum*, *Asplenium caudatum*, *Asplenium celtidifolium* (Kunze), *Asplenium ceratolepis*, *Asplenium changputungense*, *Asplenium chaseanum*, *Asplenium cheilosorum*, *Asplenium chengkouense*, *Asplenium chihuahuaense*, *Asplenium chimantae*, *Asplenium chimborazense*, *Asplenium chingianum*, *Asplenium chlorophyllum*, *Asplenium chondrophyllum*, *Asplenium cicutarium*, *Asplenium cicutarium* var. *paleaceum*, *Asplenium cirrhatum*, *Asplenium cladolepton*, *Asplenium claussenii*, *Asplenium coenobiale*, *Asplenium commutatum*, *Asplenium congestum*, *Asplenium conquisitum*, *Asplenium consimile*, *Asplenium contiguum*, *Asplenium contiguum* var. *hirtulum*, *Asplenium corderoi*, *Asplenium cordovense*, *Asplenium coriaceum*, *Asplenium coriifolium*, *Asplenium correardii*, *Asplenium costale*, *Asplenium costale* var. *robustum*, *Asplenium cowanii*, *Asplenium crenulatoserrulatum*, *Asplenium crenulatum*, *Asplenium crinicaule*, *Asplenium crinulosum*, *Asplenium cristatum*, *Asplenium cryptolepis* Fernald, *Asplenium cultrifolium* L., *Asplenium cuneatifolium*, *Asplenium cuneatum*, *Asplenium curvatum*, *Asplenium cuspidatum*, *Asplenium cuspidatum* var. *cuspidatum*, *Asplenium cuspidatum* var. *foeniculaceum*, *Asplenium cuspidatum* var. *triculum*, *Asplenium cuspidatum* var. *tripinnatum*, *Asplenium dalhousiae*, *Asplenium dareoides*, *Asplenium davallioides*, *Asplenium davisii*, *Asplenium debile*, *Asplenium debile*, *Asplenium decussatum*, *Asplenium delavayi*, *Asplenium delicatulum*, *Asplenium delicatulum* var. *cocosensis*, *Asplenium delitescens*, *Asplenium delitescens* X *laetum*, *Asplenium densum*, *Asplenium dentatum* L., *Asplenium dentatum* L., *Asplenium depauperatum*, *Asplenium deqenense*, *Asplenium dianae*, *Asplenium difforme*, *Asplenium dilatatum*, *Asplenium dimidiatum*, *Asplenium dimidiatum* var. *boliviense*, *Asplenium diplazisorum*, *Asplenium dissectum*, *Asplenium distans*, *Asplenium divaricatum*, *Asplenium divergens*, *Asplenium divisissimum*, *Asplenium doederleinii*, *Asplenium donnell-smithii*, *Asplenium dregeanum*, *Asplenium dulongjiangense*, *Asplenium duplicatoserratum*, *Asplenium eatonii*, *Asplenium ebeneum*, *Asplenium ebenoides*, *Asplenium ecuadorensis*, *Asplenium eggertii*, *Asplenium emarginatum*, *Asplenium enatum*, *Asplenium ensiforme* fo. *bicuspe*, *Asplenium ensiforme* fo. *ensiforme*, *Asplenium ensiforme* fo. *stenophyllum*, *Asplenium ensiforme*, *Asplenium erectum* var. *erectum*, *Asplenium erectum* var. *gracile*, *Asplenium erectum* var. *usambarensis*, *Asplenium erectum* var. *zeyheri*, & *Asplenium erosum* L., *Asplenium escaleroense*, *Asplenium esculentum*, *Asplenium eutecnum*, *Asplenium excelsum*, *Asplenium excisum*, *Asplenium exiguum*, *Asplenium extensum*, *Asplenium falcatum*, *Asplenium falcinellum*, *Asplenium faurei*, *Asplenium feei*, *Asplenium fengyangshanense*, *Asplenium ferulaceum*, *Asplenium fibrillosum*, *Asplenium filix-femina*, *Asplenium finckii*, *Asplenium finlaysonianum*, *Asplenium flabellulatum*, *Asplenium flabellulatum* var. *flabellulatum*, *Asplenium flabellulatum* var. *partitum*, *Asplenium flaccidum*, *Asplenium flavescens*, *Asplenium flavidum*, *Asplenium flexuosum*, *Asplenium fluminense*, *Asplenium foeniculaceum*, *Asplenium formosanum*, *Asplenium formosum* var. *carolinum*, *Asplenium formosum* var. *incultum*, *Asplenium formosum*,

Asplenium fournieri, *Asplenium fragile*, *Asplenium fragile* var. *lomense*, *Asplenium fragrans*, *Asplenium fragrans* var. *foeniculaceum*, *Asplenium franconis* var. *gracile*, *Asplenium fraxinifolium*, *Asplenium friesiorum*, *Asplenium friesiorum* var. *nesophilum*, *Asplenium fugax*, *Asplenium fujianense*, *Asplenium furcatum*, *Asplenium furfuraceum*, *Asplenium fuscipes*, *Asplenium fuscopubescens*, *Asplenium galeottii*, *Asplenium gautieri*, *Asplenium gemmiferum*, *Asplenium gentryi*,
5 *Asplenium geppii*, *Asplenium ghiesbreghtii*, *Asplenium gilliesii*, *Asplenium gilpinae*, *Asplenium glanduliserratum*, *Asplenium glenniei*, *Asplenium goldmannii*, *Asplenium gomezianum*, *Asplenium grande*, *Asplenium grandifolium*, *Asplenium grandifrons*, *Asplenium gregoriae*, *Asplenium griffithianum*, *Asplenium gulingense*, *Asplenium hainanense*, *Asplenium hallbergii*, *Asplenium hallei*, *Asplenium hallii*, *Asplenium hangzhouense*, *Asplenium haplophyllum*, *Asplenium harpeodes*,
10 *Asplenium harpeodes* var. *glaucovirans*, *Asplenium harpeodes* var. *incisum*, *Asplenium harrisii* Jenman, *Asplenium harrisonii*, *Asplenium hastatum*, *Asplenium hebeiense*, *Asplenium hemionitideum*, *Asplenium hemitomum*, *Asplenium henryi*, *Asplenium herpetopteris*, *Asplenium herpetopteris* var. *herpetopteris*, *Asplenium herpetopteris* var. *acutipinnata*,
15 *Asplenium herpetopteris* var. *masoulae*, *Asplenium herpetopteris* var. *villosum*, *Asplenium hesperium*, *Asplenium heterochroum*, *Asplenium hians*, *Asplenium hians* var. *pallenscens*, *Asplenium hoffmannii*, *Asplenium holophlebium*, *Asplenium hondoense*, *Asplenium horridum*, *Asplenium hostmannii*, *Asplenium humistratum*, *Asplenium hypomelas*, *Asplenium inaequilaterale*, *Asplenium incisum*, *Asplenium incurvatum*, *Asplenium indicum*, *Asplenium indicum* var. *indicum*,
20 *Asplenium indicum* var. *yoshingagae*, *Asplenium induratum*, *Asplenium indusiatum*, *Asplenium inexpectatum*, *Asplenium insigne*, *Asplenium insititium*, *Asplenium insolitum*, *Asplenium integerrimum*, *Asplenium interjectum*, *Asplenium jamesonii*, *Asplenium jaundeense*, *Asplenium juglandifolium*, *Asplenium kangdingense*, *Asplenium kansuense*, *Asplenium kassneri*, *Asplenium kauffussii*, *Asplenium kellermanii*, *Asplenium kentuckiense*, *Asplenium khullarii*, *Asplenium kiangsuense*,
25 *Asplenium kunzeanum*, *Asplenium lacerum*, *Asplenium laciniatum*, *Asplenium laciniatum* var. *acutipinna*, *Asplenium laciniatum* var. *laciniatum*, *Asplenium laetum* fo. *minor*, *Asplenium laetum*, *Asplenium laetum* var. *incisoserratum*, *Asplenium lamprocaulon*, *Asplenium laserpitiifolium* var. *morrisonense*, *Asplenium lastii*, *Asplenium latedens*, *Asplenium latifolium*, *Asplenium laui*, *Asplenium laurentii*, *Asplenium leandrianum*, *Asplenium lechleri*, *Asplenium leiboense*, *Asplenium lepidorachis*, *Asplenium leptochlamys*, *Asplenium leptophyllum*, *Asplenium levyi*, *Asplenium lindbergii*, *Asplenium lindenii*,
30 *Asplenium lineatum*, *Asplenium lividum*, *Asplenium lobatum*, *Asplenium lobulatum*, *Asplenium lokohoense*, *Asplenium longicauda*, *Asplenium longicaudatum*, *Asplenium longifolium*, *Asplenium longisorum*, *Asplenium longjiense*, *Asplenium lorentzii*, *Asplenium loriceum*, *Asplenium loxogrammoides*, *Asplenium lugubre*, *Asplenium lunulatum*, *Asplenium lunulatum* var. *pteropus*, *Asplenium lushanense*, *Asplenium lydgatei*, *Asplenium macilentum*, *Asplenium macraei*, *Asplenium macrodictyon*, *Asplenium macrophlebium*, *Asplenium macrophyllum*, *Asplenium macropterum*, *Asplenium macrosorum*,
35 *Asplenium macrotis*, *Asplenium macrurum*, *Asplenium mainlingense*, *Asplenium mangindranense*, *Asplenium manni*, *Asplenium marginatum* L., *Asplenium marojejense*, *Asplenium martianum*, *Asplenium matsumurae*, *Asplenium mauritiensis* Lorence, *Asplenium maximum*, *Asplenium*, ii, *Asplenium megalura*, *Asplenium megaphyllum*, *Asplenium meiotomum*, *Asplenium melanopus*, *Asplenium membranifolium*, *Asplenium meniscioides*, *Asplenium mesosorum*, *Asplenium mexicanum*, *Asplenium micropaleatum*, *Asplenium microtum*, *Asplenium mildbraedii*, *Asplenium mildei*, *Asplenium minimum*, *Asplenium minutum*, *Asplenium miradoreense*, *Asplenium miyunense*, *Asplenium mocce-*
40 *nianum*, *Asplenium mocquersii*, *Asplenium modestum*, *Asplenium monanthes* var. *menziesii*, *Asplenium monanthes* L., *Asplenium monanthes* var. *monanthes*, *Asplenium monanthes* var. *castaneum*, *Asplenium monanthes* var. *wagneri*, *Asplenium monanthes* var. *yungense*, *Asplenium monodon*, *Asplenium montanum*, *Asplenium mosetenense*, *Asplenium moupinense*, *Asplenium mucronatum*, *Asplenium munchii*, *Asplenium muticum*, *Asplenium myapteron*, *Asplenium myriophyllu*,
45 *Asplenium nakanoanum*, *Asplenium nanchuanense*, *Asplenium nemorale*, *Asplenium neolaserpitiifolium*, *Asplenium neomutijugum*, *Asplenium neovarians*, *Asplenium nesii*, *Asplenium nesioticum*, *Asplenium nidus* L., *Asplenium nigricans*, *Asplenium niponicum*, *Asplenium normale*, *Asplenium normale* var. *angustum*, *Asplenium obesum*, *Asplenium oblongatum*, *Asplenium oblongifolium*, *Asplenium obovatum*, *Asplenium obscurum*, *Asplenium obscurum* var. *angustum*,
50 *Asplenium obtusatum* var. *obtusatum*, *Asplenium obtusatum* var. *sphenoides*, *Asplenium obtusifolium* L., *Asplenium obtusissimum*, *Asplenium obversum*, *Asplenium ochraceum*, *Asplenium oellgaardii*, *Asplenium ofeliae*, *Asplenium oldhami*, *Asplenium oligosorum*, *Asplenium olivaceum*, *Asplenium onopteris* L., *Asplenium onustum*, *Asplenium ortegae*, *Asplenium otites*, *Asplenium palaciosii*, *Asplenium palmeri*, *Asplenium partitum*, *Asplenium parvisorum*, *Asplenium parviusculum*, *Asplenium parvulum*, *Asplenium patens*, *Asplenium paucifolium*, *Asplenium paucijugum*, *Asplenium pauciv-*
55 *enosum*, *Asplenium pearcei*, *Asplenium pekinense*, *Asplenium pellucidum*, *Asplenium pendulum*, *Asplenium petiolulatum*, *Asplenium phyllitidis*, *Asplenium pimpinellifolium*, *Asplenium pinnatifidum*, *Asplenium pinnatum*, *Asplenium platyneuron*, *Asplenium platyneuron* var. *bacculum-rubrum*, *Asplenium platyneuron* var. *incisum*, *Asplenium platyphyllum*, *Asplenium plumbeum*, *Asplenium poloense*, *Asplenium polymeris*, *Asplenium polymorphum*, *Asplenium polyodon*, *Asplenium polyodon* var. *knudsenii*, *Asplenium polyodon* var. *nitidulum*, *Asplenium polyodon* var. *sectum*, *Asplenium polyodon* var. *subcaudatum*, *Asplenium polyphyllum*, *Asplenium poolii*, *Asplenium poolii* fo. *simplex*, *Asplenium poolii* var. *linearipinnatum*, *Asplenium potosinum*, *Asplenium potosinum* var. *incisum*, *Asplenium praegracile*, *Asplenium praemorsum*, *Asplenium preussii*, *Asplenium pringleanum*, *Asplenium pringlei*, *Asplenium prionitis*, *Asplenium procerum*, *Asplenium progrediens*, *Asplenium projectum*, *Asplenium prolongatum*, *Asplenium propinquum*, *Asplenium protensum*, *Asplenium pseudoangustum*, *Asplenium pseudoerectum*, *Asplenium pseudofontanum*, *Asplenium pseudolaserpitiifo-*

lium, *Asplenium pseudonormale*, *Asplenium pseudopellucidum*, *Asplenium pseudopraemorsum*, *Asplenium pseudovarians*, *Asplenium pseudowilfordii*, *Asplenium pseudowrightii*, *Asplenium psilacrum*, *Asplenium pteropus*, *Asplenium pubirhizoma*, *Asplenium pulchellum*, *Asplenium pulchellum* var. *subhorizontale*, *Asplenium pulcherrimum*, *Asplenium pulicosum*, *Asplenium pulicosum* var. *maius*, *Asplenium pululahuae*, *Asplenium pumilum*, *Asplenium pumilum* var. *hymenophylloides*, *Asplenium pumilum* var. *laciniatum*, *Asplenium purdieanum*, *Asplenium purpurascens*, *Asplenium pyramidatum*, *Asplenium qiujiangense*, *Asplenium quercicola*, *Asplenium quitense*, *Asplenium raddianum*, *Asplenium radiatum*, *Asplenium radicans* L., *Asplenium radicans*, *Asplenium radicans* var. *costaricense*, *Asplenium radicans* var. *partitum*, *Asplenium radicans* var. *radicans*, *Asplenium radicans* var. *uniseriale*, *Asplenium recumbens*, *Asplenium reflexum*, *Asplenium regulare* var. *latior*, *Asplenium repandulum*, *Asplenium repens*, *Asplenium repente*, *Asplenium resiliens*, *Asplenium retusulum*, *Asplenium rhipidoneuron*, *Asplenium rhizophorum* L., *Asplenium rhizophyllum*, *Asplenium rhizophyllum* L., *Asplenium rhizophyllum* var. *proliferum*, *Asplenium rhomboideum*, *Asplenium rigidum*, *Asplenium riparium*, *Asplenium rivale*, *Asplenium rockii*, *Asplenium roemerianum*, *Asplenium roemerianum* var. *mindensis*, *Asplenium rosenstockianum*, *Asplenium rubinum*, *Asplenium ruizianum*, *Asplenium rusbyanum*, *Asplenium ruta-muraria* L., *Asplenium ruta-muraria* var. *cryptolepis*, *Asplenium rutaceum*, *Asplenium rutaceum* var. *disculiferum*, *Asplenium rutaefolium*, *Asplenium rutifolium*, *Asplenium salicifolium* L., *Asplenium salicifolium* var. *aequilaterale*, *Asplenium salicifolium* var. *salicifolium*, *Asplenium sampsoni*, *Asplenium sanchezii*, *Asplenium sanderi*, *Asplenium sandersonii*, *Asplenium sanguinolentum*, *Asplenium sarelii*, *Asplenium sarelii* var. *magnum*, *Asplenium sarelii* var. *sarelii*, *Asplenium saxicola*, *Asplenium scalifolium*, *Asplenium scandicinum*, *Asplenium schizophyllum*, *Asplenium schkuhrii*, *Asplenium sciadophilum*, *Asplenium scolopendrium* L., *Asplenium scortechinii*, *Asplenium seileri*, *Asplenium semipinnatum*, *Asplenium septentrionale*, *Asplenium serra*, *Asplenium serra* var. *imrayanum*, *Asplenium serratissimum*, *Asplenium serratum* L., *Asplenium serratum* var. *caudatum*, *Asplenium serricula*, *Asplenium sessilifolium*, *Asplenium sessilifolium* var. *guatemalense*, *Asplenium sessilifolium* var. *minus*, *Asplenium sessilifolium* var. *occidentale*, *Asplenium sessilipinum*, *Asplenium setosum*, *Asplenium shepherdii*, *Asplenium shepherdii* var. *bipinnatum*, *Asplenium shepherdii* var. *flagelliferum*, *Asplenium shokianum*, *Asplenium simii*, *Asplenium simonsianum*, *Asplenium sintenisii*, *Asplenium skinneri*, *Asplenium skinneri*, *Asplenium sodiroi*, *Asplenium soleiolioides*, *Asplenium solidum* var. *stenophyllum*, *Asplenium solmsii*, *Asplenium* sp.-N.-Halle-2234, *Asplenium spathulinum*, *Asplenium spectabile*, *Asplenium speluncae*, *Asplenium sphaerosporum*, *Asplenium sphenotomum*, *Asplenium spinescens*, *Asplenium splendens*, *Asplenium sprucei*, *Asplenium squamosum* L., *Asplenium standleyi*, *Asplenium stellatum*, *Asplenium stenocarpum*, *Asplenium stoloniferum*, *Asplenium stolonipes*, *Asplenium striatum* L., *Asplenium stuebelianum*, *Asplenium stuhlmannii*, *Asplenium suave*, *Asplenium subalatum*, *Asplenium subcrenatum*, *Asplenium subdigitatum*, *Asplenium subdimidiatum*, *Asplenium subintegrum*, *Asplenium sublaesepitiifolium*, *Asplenium sublongum*, *Asplenium subnudum*, *Asplenium suborbiculare*, *Asplenium subtenuifolium*, *Asplenium subtile*, *Asplenium subtoramanum*, *Asplenium subtrapezoideum*, *Asplenium subvarians*, *Asplenium sulcatum*, *Asplenium sylvaticum*, *Asplenium szechuanense*, *Asplenium taiwanense*, *Asplenium tenerrimum*, *Asplenium tenerum*, *Asplenium tenuicaule*, *Asplenium tenuifolium*, *Asplenium tenuifolium* var. *minor*, *Asplenium tenuifolium* var. *tenuifolium*, *Asplenium tenuissimum*, *Asplenium ternatum*, *Asplenium theciferum*, *Asplenium theciferum* var. *concinnum*, *Asplenium thunbergii*, *Asplenium tianmushanense*, *Asplenium tianshanense*, *Asplenium tibeticum*, *Asplenium tocoraniense*, *Asplenium toramanum*, *Asplenium trapezoideum*, *Asplenium tricholepis*, *Asplenium trichomanes* L., *Asplenium trichomanes* subsp. *inexpectans*, *Asplenium trichomanes* subsp. *quadrivalens*, *Asplenium trichomanes* subsp. *trichomanes*, *Asplenium trichomanes* var. *harovii*, *Asplenium trichomanes* var. *herbaceum*, *Asplenium trichomanes* var. *repens*, *Asplenium trichomanes* var. *viridissimum*, *Asplenium trichomanes-dentatum* L., *Asplenium trigonopterum*, *Asplenium trilobatum*, *Asplenium trilobum*, *Asplenium triphyllum*, *Asplenium triphyllum* var. *compactum*, *Asplenium triphyllum* var. *gracillimum*, *Asplenium triphyllum* var. *herbaceum*, *Asplenium tripteropus*, *Asplenium triquetrum*, *Asplenium truncorum*, *Asplenium tsaratananense*, *Asplenium tucumanense*, *Asplenium tuerckheimii*, *Asplenium tunquiniense*, *Asplenium ulbrichtii*, *Asplenium ultimum*, *Asplenium unilaterale*, *Asplenium unilaterale* var. *decurrens*, *Asplenium unilaterale* var. *udum*, *Asplenium unilaterale* var. *unilaterale*, *Asplenium uniseriale*, *Asplenium uropterum*, *Asplenium vagans*, *Asplenium vareschianum*, *Asplenium variabile* var. *paucijugum*, *Asplenium variabile* var. *variabile*, *Asplenium varians* subsp. *fimbriatum*, *Asplenium varians*, *Asplenium vastum*, *Asplenium venturae*, *Asplenium venulosum*, *Asplenium verapax*, *Asplenium vesiculosum*, *Asplenium vespertinum*, *Asplenium villosum*, *Asplenium virens*, *Asplenium viride*, *Asplenium viridifrons*, *Asplenium virillae*, *Asplenium viviparioides*, *Asplenium viviparum*, *Asplenium viviparum* var. *viviparum*, *Asplenium viviparum* var. *lineatu*, *Asplenium volubile*, *Asplenium vulcanicum*, *Asplenium wacketii*, *Asplenium wagneri*, *Asplenium wallichianum*, *Asplenium warneckei*, *Asplenium wilfordii*, *Asplenium williamsii*, *Asplenium wrightii*, *Asplenium wrightioides*, *Asplenium wuliangshanense*, *Asplenium xianqianense*, *Asplenium xinjiangense*, *Asplenium xinyiense*, *Asplenium yelagagense*, *Asplenium yoshinagae*, *Asplenium yunnanense*, *Asplenium zamiifolium*, *Asplenium zanzibaricum*, *Asplenium biscayneanum*, *Asplenium curtissii*, *Asplenium ebenoides*, *Asplenium herb-wagneri*, *Asplenium heteroresiliens*, *Asplenium kenzoii*, *Asplenium plenum*, *Asplenium wangii*, and *Asplenium* × *clermontiae*, *Asplenium* × *gravesii*.

[0153] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Blechnaceae, Genus *Blechnum* L. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Blechnaceae, Genus *Blechnum* L. selected from but not limited to *Blechnum amabile*, *Blechnum*

num appendiculatum, *Blechnum articulatum*, *Blechnum australe*, *Blechnum austrobrasilianum*, *Blechnum binervatum*, *Blechnum blechnoides*, *Blechnum brasiliense*, *Blechnum capense*, *Blechnum cartilagineum*, *Blechnum castaneum*, *Blechnum chambersii*, *Blechnum chilense*, *Blechnum colensoi*, *Blechnum contiguum*, *Blechnum cordatum*, *Blechnum coriaceum*, *Blechnum discolor*, *Blechnum doodioides*, *Blechnum durum*, *Blechnum eburneum*, *Blechnum ensiforme*,
 5 *Blechnum filiforme*, *Blechnum fluviatile*, *Blechnum fragile*, *Blechnum fraseri*, *Blechnum fullagari*, *Blechnum gibbum*, *Blechnum glandulosum*, *Blechnum gracile*, *Blechnum hancockii*, *Blechnum hastatum*, *Blechnum howeanum*, *Blechnum indicum*, *Blechnum kunthianum*, *Blechnum laevigatum*, *Blechnum loxense*, *Blechnum magellanicum*, *Blechnum membranaceum*, *Blechnum microbasis*, *Blechnum microphyllum*, *Blechnum milnei*, *Blechnum minus*, *Blechnum mochaenum*,
 10 *Blechnum montanum*, *Blechnum moorei*, *Blechnum moritzianum*, *Blechnum nigrum*, *Blechnum niponicum*, *Blechnum norfolkianum*, *Blechnum novae-zelandiae*, *Blechnum nudum*, *Blechnum obtusatum*, *Blechnum occidentale*, *Blechnum oceanicum*, *Blechnum orientale*, *Blechnum patersonii*, *Blechnum penna-marina*, *Blechnum polypodioides*, *Blechnum procerum*, *Blechnum punctulatum*, *Blechnum sampaioanum*, *Blechnum schiedeanum*, *Blechnum schomburgkii*, *Blechnum serrulatum*, *Blechnum simillimum*, *Blechnum spicant*, *Blechnum stipitellatum*, *Blechnum tabulare*, *Blechnum triangularifolium*, *Blechnum vieillardii*, *Blechnum vulcanicum*, *Blechnum wattsii*, *Blechnum whelanii*, and *Blechnum wurunuran*.

[0154] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Dryopteridaceae* Genus *Acrophorus*, Genus *Acroromohra*, Genus *Anapausia*, Genus *Arachniodes*, Genus *Bolbitis*, Genus *Ctenitis*, Genus *Cyclodium*, Genus *Cyrtogonellum*, Genus *Cyrtomidictyum*, Genus *Cyrtomium*, Genus *Dicalpe*, Genus *Didymochlaena*, Genus *Dryopsis*, Genus *Dryopteris*, Genus *Elaphoglossum*, Genus *Hypodematium*, Genus *Lastreopsis*, Genus *Leptorumohra*, Genus *Leucostegia*, Genus *Lithostegia*, Genus *Lomagramma*, Genus *Maxonia*, Genus *Megalastrum*, Genus *Olfersia*, Genus *Peranema*, Genus *Phanerophlebia*, Genus *Phanerophlebiopsis*, Genus *Polybotrya*, Genus *Polystichopsis*, Genus *Polystichum*, Genus *Rumohra*, Genus *Sorolepidium*, Genus *Stigmatopteris* or Genus *Teratophyllum*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Dryopteridaceae*, Genus *Bolbitis*, selected from but not limited to *Bolbitis acrostichoides*, *Bolbitis aliena*, *Bolbitis angustipinna*, *Bolbitis appendiculata*, *Bolbitis auriculata*, *Bolbitis bernoullii*, *Bolbitis bipinnatifida*, *Bolbitis cadieri*, *Bolbitis christensenii*, *Bolbitis confertifolia*, *Bolbitis costata*, *Bolbitis crispatula*, *Bolbitis fluviatilis*, *Bolbitis gaboonensis*, *Bolbitis gemmifera*, *Bolbitis hainanensis*, *Bolbitis hastata*, *Bolbitis hekouensis*, *Bolbitis hemiotis*, *Bolbitis heteroclita*, *Bolbitis heudelotii*, *Bolbitis humblotii*, *Bolbitis interlineata*, *Bolbitis latipinna*, *Bolbitis laxireticulata*, *Bolbitis lindigii*, *Bolbitis lonchophora*, *Bolbitis longiflagellata*, *Bolbitis major*, *Bolbitis media*, *Bolbitis nicotianifolia*, *Bolbitis nodiflora*, *Bolbitis novoguineensis*, *Bolbitis oligarchica*, *Bolbitis palustris*, *Bolbitis pandurifolia*, *Bolbitis pergamentacea*, *Bolbitis portoricensis*, *Bolbitis presliana*, *Bolbitis quoyana*, *Bolbitis rawsonii*, *Bolbitis repanda*, *Bolbitis rhizophylla*, *Bolbitis riparia*, *Bolbitis rivularis*, *Bolbitis sagenioides*, *Bolbitis salicina*, *Bolbitis scalpturata*, *Bolbitis scandens*, *Bolbitis semicordata*, *Bolbitis semipinnatifida*, *Bolbitis serrata*, *Bolbitis serratifolia*, *Bolbitis simplex*, *Bolbitis sinensis*, *Bolbitis singaporensis*, *Bolbitis sinuata*, *Bolbitis subcordata*, *Bolbitis subcrenata*, *Bolbitis taylorii*, *Bolbitis tibetica*, *Bolbitis tonkinensis*, *Bolbitis umbrosa*, *Bolbitis vanuaensis*, and *Bolbitis virens*.

[0155] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Lomariopsidaceae*, Genus *Nephrolepis*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Lomariopsidaceae*, Genus *Nephrolepis* is selected from but not limited to *Nephrolepis abrupta*, *Nephrolepis acuminata*, *Nephrolepis acutifolia*, *Nephrolepis arida*, *Nephrolepis arthropteroides*, *Nephrolepis biserrata* var. *auriculata*, *Nephrolepis brownii*, *Nephrolepis celebica*, *Nephrolepis clementis*, *Nephrolepis cordifolia*, *Nephrolepis davalliae*, *Nephrolepis davallioides*, *Nephrolepis dayakorum*, *Nephrolepis delicatula*, *Nephrolepis dicksonioides*, *Nephrolepis duffii*, *Nephrolepis exaltata* ssp. *exaltata* ssp. *Hawaiiensis*, *Nephrolepis falcata*, *Nephrolepis falciformis*, *Nephrolepis glabra*, *Nephrolepis hirsutula*, *Nephrolepis humatoides*, *Nephrolepis iridescens*, *Nephrolepis kurotawae*, *Nephrolepis laurifolia*, *Nephrolepis lauterbachii*, *Nephrolepis lindsayae*, *Nephrolepis multifida*, *Nephrolepis multiflora*,
 45 *Nephrolepis niphoboloides*, *Nephrolepis obliterate*, *Nephrolepis paludosa*, *Nephrolepis pectinata*, *Nephrolepis pendula*, *Nephrolepis persicifolia*, *Nephrolepis pickelii*, *Nephrolepis pilosula*, *Nephrolepis pubescens*, *Nephrolepis pumicicola*, *Nephrolepis radicans*, *Nephrolepis rivularis*, *Nephrolepis rosenstockii*, *Nephrolepis saligna*, *Nephrolepis schlechteri*, *Nephrolepis serrate*, *Nephrolepis thomsoni*, *Nephrolepis undulata* var. *aureoglandulosa*, *Nephrolepis x averyi*., *Nephrolepis x copelandii*, and *Nephrolepis x medlerae*.

[0156] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae*, Genus *Campyloneurum*, Genus *Drynaria*, Genus *Lepisorus*, Genus *Microgramma*, Genus *Microsorium*, Genus *Neurodium*, Genus *Niphidium*, Genus *Pecluma* M.G., Genus *Phlebodium*, Genus *Phymatosorus*, Genus *Platyserium*, Genus *Pleopeltis*, Genus *Polypodium* L.

[0157] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae*, Genus *Polypodium* L. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family *Polypodiaceae*, Genus *Polypodium* L. selected from but not limited to *Polypodium ab-sidatum*, *Polypodium acutifolium*, *Polypodium adiantiforme*, *Polypodium aequale*, *Polypodium affine*, *Polypodium albidopaleatum*, *Polypodium alcicorne*, *Polypodium alfarii*, *Polypodium alfredii*, *Polypodium alfredii* var. *curtii*, *Polypodium*

allosuroides, *Polypodium alsophilicola*, *Polypodium amamanum*, *Polypodium amoenum*, *Polypodium amorphum*, *Polypodium anetioides*, *Polypodium anfractuosum*, *Polypodium anguinum*, *Polypodium angustifolium* f. *remotifolia*, *Polypodium angustifolium* var. *amphostenon*, *Polypodium angustifolium* var. *heterolepis*, *Polypodium angustifolium* var. *monstrosa*, *Polypodium angustipaleatum*, *Polypodium angustissimum*, *Polypodium anisomeron* var. *pectinatum*, *Polypodium antioquianum*, *Polypodium aoristorum*, *Polypodium apagolepis*, *Polypodium apicidens*, *Polypodium apiculatum*, *Polypodium apoense*, *Polypodium appalachianum*, *Polypodium appressum*, *Polypodium arenarium*, *Polypodium argentinum*, *Polypodium argutum*, *Polypodium armatum*, *Polypodium aromaticum*, *Polypodium aspersum*, *Polypodium assurgens*, *Polypodium atrum*, *Polypodium auriculatum*, *Polypodium balaonense*, *Polypodium balliviani*, *Polypodium bamleri*, *Polypodium bangii*, *Polypodium bartlettii*, *Polypodium basale*, *Polypodium bernoullii*, *Polypodium biauratum*, *Polypodium bifrons*, *Polypodium blepharodes*, *Polypodium bolivari*, *Polypodium bolivianum*, *Polypodium bolobense*, *Polypodium bombycinum*, *Polypodium bombycinum* var. *insularum*, *Polypodium bradeorum*, *Polypodium bryophilum*, *Polypodium bryopodium*, *Polypodium buchtienii*, *Polypodium buesii*, *Polypodium bulbotrichum*, *Polypodium caceresii*, *Polypodium californicum* f. *brauscombii*, *Polypodium californicum* f. *parsonsiae*, *Polypodium californicum*, *Polypodium calophlebium*, *Polypodium calvum*, *Polypodium camptophyllum* var. *abbreviatum*, *Polypodium capitellatum*, *Polypodium carpintera*, *Polypodium chachapoyense*, *Polypodium chartaceum*, *Polypodium chimantense*, *Polypodium chiricanum*, *Polypodium choquetangense*, *Polypodium christensenii*, *Polypodium christii*, *Polypodium chrysotrichum*, *Polypodium cilirolepis*, *Polypodium cinerascens*, *Polypodium collinsii*, *Polypodium colysoides*, *Polypodium confluens*, *Polypodium conforme*, *Polypodium confusum*, *Polypodium congregatifolium*, *Polypodium connellii*, *Polypodium consimile* var. *bourgaeum*, *Polypodium consimile* var. *minor*, *Polypodium conterminans*, *Polypodium contiguum*, *Polypodium cookii*, *Polypodium coriaceum*, *Polypodium coronans*, *Polypodium costaricense*, *Polypodium costatum*, *Polypodium crassifolium* f. *angustissimum*, *Polypodium crassifolium* var. *longipes*, *Polypodium crassulum*, *Polypodium craterisorum*, *Polypodium cryptum*, *Polypodium crystalloneuron*, *Polypodium cucullatum* var. *planum*, *Polypodium cuencanum*, *Polypodium cumingianum*, *Polypodium cupreolepis*, *Polypodium curranii*, *Polypodium curvans*, *Polypodium cyathicola*, *Polypodium cyathisorum*, *Polypodium cyclocolpon*, *Polypodium daguense*, *Polypodium damunense*, *Polypodium dareiformioides*, *Polypodium dasyleura*, *Polypodium decipiens*, *Polypodium decorum*, *Polypodium delicatulum*, *Polypodium deltoideum*, *Polypodium demeraranum*, *Polypodium denticulatum*, *Polypodium diaphanum*, *Polypodium dilatatum*, *Polypodium dispersum*, *Polypodium dissectum*, *Polypodium dissimulans*, *Polypodium dolichosorum*, *Polypodium dolorense*, *Polypodium donnell-smithii*, *Polypodium drymoglossoides*, *Polypodium ebeninum*, *Polypodium eggersii*, *Polypodium elmeri*, *Polypodium elongatum*, *Polypodium enterosoroides*, *Polypodium erubescens*, *Polypodium erythrolepis*, *Polypodium erythrotrichum*, *Polypodium eurybasis*, *Polypodium eurybasis* var. *villosum*, *Polypodium exornans*, *Polypodium falcoideum*, *Polypodium fallacissimum*, *Polypodium farinosum*, *Polypodium faucium*, *Polypodium feei*, *Polypodium ferrugineum*, *Polypodium feuillei*, *Polypodium firmulum*, *Polypodium firmum*, *Polypodium flaccidum*, *Polypodium flagellare*, *Polypodium flexuosum*, *Polypodium flexuosum* var. *ekmanii*, *Polypodium forbesii*, *Polypodium formosanum*, *Polypodium fraxinifolium* subsp. *articulatum*, *Polypodium fraxinifolium* subsp. *luridum*, *Polypodium fructuosum*, *Polypodium fucoides*, *Polypodium fulvescens*, *Polypodium galeottii*, *Polypodium glaucum*, *Polypodium glycyrrhiza*, *Polypodium gracillimum*, *Polypodium gramineum*, *Polypodium grandifolium*, *Polypodium gratum*, *Polypodium graveolens*, *Polypodium griseo-nigrum*, *Polypodium griseum*, *Polypodium guttatum*, *Polypodium haallioanum*, *Polypodium hammatisorum*, *Polypodium hancockii*, *Polypodium haplophlebicum*, *Polypodium harrisii*, *Polypodium hastatum* var. *simplex*, *Polypodium hawaiiense*, *Polypodium heanophyllum*, *Polypodium helleri*, *Polypodium hemionitidium*, *Polypodium henryi*, *Polypodium herzogii*, *Polypodium hesperium*, *Polypodium hessii*, *Polypodium hombersleyi*, *Polypodium hostmannii*, *Polypodium humile*, *Polypodium hyalinum*, *Polypodium iboense*, *Polypodium induens* var. *subdentatum*, *Polypodium insidiosum*, *Polypodium insigne*, *Polypodium intermedium* subsp. *masafueranum* var. *obtusesserratum*, *Polypodium intramarginale*, *Polypodium involutum*, *Polypodium itatiayense*, *Polypodium javanicum*, *Polypodium juglandifolium*, *Polypodium kaniense*, *Polypodium knowltoniorum*, *Polypodium kyimbilense*, *Polypodium l'herminieri* var. *costaricense*, *Polypodium lachniferum* f. *incurvata*, *Polypodium lachniferum* var. *glabrescens*, *Polypodium lachnopus*, *Polypodium lanceolatum* var. *complanatum*, *Polypodium lanceolatum* var. *trichophorum*, *Polypodium latevagans*, *Polypodium laxifrons*, *Polypodium laxifrons* var. *lividum*, *Polypodium lehmannianum*, *Polypodium leiorhizum*, *Polypodium leptopodon*, *Polypodium leuconeuron* var. *angustifolia*, *Polypodium leuconeuron* var. *latifolium*, *Polypodium leucosticta*, *Polypodium limulum*, *Polypodium lindigii*, *Polypodium lineatum*, *Polypodium lomarioides*, *Polypodium longifrons*, *Polypodium lorentense*, *Polypodium loriceum* var. *umbraticum*, *Polypodium loriforme*, *Polypodium loxogramme* f. *gigas*, *Polypodium ludens*, *Polypodium luzonicum*, *Polypodium lycopodioides* f. *obtusum*, *Polypodium lycopodioides* L., *Polypodium macrolepis*, *Polypodium macrophyllum*, *Polypodium macrosorum*, *Polypodium macrosphaerum*, *Polypodium maculosum*, *Polypodium madreense*, *Polypodium manmeiense*, *Polypodium margaritifera*, *Polypodium maritimum*, *Polypodium martensii*, *Polypodium majoris*, *Polypodium megalepis*, *Polypodium melanotrichum*, *Polypodium menisciifolium* var. *pubescens*, *Polypodium meniscioides*, *Polypodium merrillii*, *Polypodium mettenii*, *Polypodium mexiae*, *Polypodium microsorum*, *Polypodium militare*, *Polypodium minimum*, *Polypodium minusculum*, *Polypodium mixtum*, *Polypodium mollendense*, *Polypodium mollissimum*, *Polypodium moniliforme* var. *minus*, *Polypodium monoides*, *Polypodium monticola*, *Polypodium montigenum*, *Polypodium moritzianum*, *Polypodium moultonii*, *Polypodium multicaudatum*, *Polypodium multilineatum*, *Polypodium multisorum*, *Polypodium*

munchii, *Polypodium muscoides*, *Polypodium myriolepis*, *Polypodium myriophyllum*, *Polypodium myriotrichum*, *Polypodium nematorhizon*, *Polypodium nemorale*, *Polypodium nesioticum*, *Polypodium nigrescentium*, *Polypodium nigripes*, *Polypodium nigrocinctum*, *Polypodium nimbatum*, *Polypodium nitidissimum*, *Polypodium nitidissimum* var. *latior*, *Polypodium nubrigenum*, *Polypodium oligolepis*, *Polypodium oligosorum*, *Polypodium oligosorum*, *Polypodium olivaceum*,
 5 *Polypodium olivaceum* var. *elatum*, *Polypodium oodes*, *Polypodium oosphaerum*, *Polypodium oreophilum*, *Polypodium ornatissimum*, *Polypodium ornatum*, *Polypodium ovatum*, *Polypodium oxylobum*, *Polypodium oxypholis*, *Polypodium pakkaense*, *Polypodium pallidum*, *Polypodium palmatopedatum*, *Polypodium palmeri*, *Polypodium panamense*, *Polypodium parvum*, *Polypodium patagonicum*, *Polypodium paucisorum*, *Polypodium pavonianum*, *Polypodium pectinatum*
 10 var. *caliense*, *Polypodium pectinatum* var. *hispidum*, *Polypodium pellucidum*, *Polypodium pendulum* var. *boliviense*, *Polypodium percrassum*, *Polypodium perpusillum*, *Polypodium peruvianum* var. *subgibbosum*, *Polypodium phyllitidis* var. *elongatum*, *Polypodium pichinchense*, *Polypodium pilosissimum*, *Polypodium pilosissimum* var. *glabriusculum*, *Polypodium pilosissimum* var. *tunguraquensis*, *Polypodium pityrolepis*, *Polypodium platyphyllum*, *Polypodium playfairii*, *Polypodium plebeium* var. *cooperi*, *Polypodium plectolepidioides*, *Polypodium pleolepis*, *Polypodium plesiosorum* var. *i*, *Polypodium podobasis*, *Polypodium podocarpum*, *Polypodium poloense*, *Polypodium polydatylon*, *Polypodium polypodioides*
 15 var. *aciculare*, *Polypodium polypodioides* var. *michauxianum*, *Polypodium praetermissum*, *Polypodium preslianum* var. *immersum*, *Polypodium procerum*, *Polypodium procerum*, *Polypodium productum*, *Polypodium productum*, *Polypodium prolongilobum*, *Polypodium propinguum*, *Polypodium proteus*, *Polypodium pruinarum*, *Polypodium pseudocapillare*, *Polypodium pseudofratrum*, *Polypodium pseudonutans*, *Polypodium pseudoserratum*, *Polypodium pulcherrimum*, *Polypodium pulogense*, *Polypodium pungens*, *Polypodium purpusii*, *Polypodium radicale*, *Polypodium randallii*, *Polypodium ratiborii*, *Polypodium reclinatum*, *Polypodium recreense*, *Polypodium repens* var. *abruptum*, *Polypodium revolvens*,
 20 *Polypodium rhachipterygium*, *Polypodium rhomboideum*, *Polypodium rigens*, *Polypodium robustum*, *Polypodium roraimense*, *Polypodium roraimense*, *Polypodium rosei*, *Polypodium rosenstockii*, *Polypodium rubidum*, *Polypodium rudimentum*, *Polypodium rusbyi*, *Polypodium sablanianum*, *Polypodium sarmentosum*, *Polypodium saxicola*, *Polypodium schenckii*, *Polypodium schlechteri*, *Polypodium scolopendria*, *Polypodium scolopendria*, *Polypodium scolopendrium*,
 25 *Polypodium scouleri*, *Polypodium scutulatum*, *Polypodium segregatum*, *Polypodium semihirsutum*, *Polypodium semihirsutum* var. *fuscetosum*, *Polypodium senile* var. *minor*, *Polypodium sericeolanatum*, *Polypodium serraeforme*, *Polypodium serricula*, *Polypodium sesquipedala*, *Polypodium sessilifolium*, *Polypodium setosum* var. *calvum*, *Polypodium setulosum*, *Polypodium shaferi*, *Polypodium sibomense*, *Polypodium siccum*, *Polypodium simacense*, *Polypodium simulans*, *Polypodium singeri*, *Polypodium sinicum*, *Polypodium sintenisii*, *Polypodium skutchii*, *Polypodium sloanei*, *Polypodium sodiroi*, *Polypodium sordidulum*, *Polypodium sordidum*, *Polypodium sphaeropteroides*, *Polypodium sphenodes*,
 30 *Polypodium sprucei*, *Polypodium sprucei* var. *furcativenosa*, *Polypodium steirolepis*, *Polypodium stenobasis*, *Polypodium stenolepis*, *Polypodium stenopterum*, *Polypodium subcapillare*, *Polypodium subflabelliforme*, *Polypodium subhemionitidum*, *Polypodium subinaequale*, *Polypodium subintegrum*, *Polypodium subspathulatum*, *Polypodium subtile*, *Polypodium subvestitum*, *Polypodium subviride*, *Polypodium superficiale* var. *attenuatum*, *Polypodium superficiale* var. *chinensis*, *Polypodium sursumcurrens*, *Polypodium tablazianum*, *Polypodium taenifolium*, *Polypodium tamandarei*, *Polypodium tatei*, *Polypodium tenuiculum* var. *acrosora*, *Polypodium tenuiculum* var. *brasiliense*, *Polypodium tenuilore*, *Polypodium tenuinerve*, *Polypodium tepuiense*, *Polypodium teresae*, *Polypodium tetragonum* var. *incompletum*, *Polypodium thysanolepis* var. *bipinnatifidum*, *Polypodium thysanolepis*, var. *thysanolepis*, *Polypodium thysanolepsi*, *Polypodium tobagense*, *Polypodium trichophyllum*, *Polypodium tridactylum*, *Polypodium tridentatum*, *Polypodium trifurcatum* var.
 40 *brevipes*, *Polypodium triglossum*, *Polypodium truncatulum*, *Polypodium truncicola* var. *major*, *Polypodium truncicola* var. *minor*, *Polypodium tuberosum*, *Polypodium tunguraguae*, *Polypodium turquinum*, *Polypodium turrialbae*, *Polypodium ursipes*, *Polypodium vagans*, *Polypodium valdealatum*, *Polypodium versteegii*, *Polypodium villagrani*, *Polypodium virginianum* f. *cambroideum*, *Polypodium virginianum* f. *periferens*, *Polypodium vittarioides*, *Polypodium vulgare*, *Polypodium vulgare* L., *Polypodium vulgare* subsp. *oreophilum*, *Polypodium vulgare* var. *acuminatum*, *Polypodium vulpinum*,
 45 *Polypodium williamsii*, *Polypodium wobbenense*, *Polypodium x fallacissimum-guttatum*, *Polypodium xantholepis*, *Polypodium xiphopteris*, *Polypodium yarumalense*, *Polypodium yungense*, and *Polypodium zosteriforme*.

[0158] In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platycterium*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Order Polypodiales, Family Polypodiaceae, Genus *Platycterium* selected from but not limited to *Platycterium alcorni*,
 50 *Platycterium andinum*, *Platycterium angolense*, *Platycterium bifurcatum*, *Platycterium coronarium*, *Platycterium elephantotis*, *Platycterium ellisii*, *Platycterium grande*, *Platycterium hillii*, *Platycterium holttumii*, *Platycterium madagascariense*, *Platycterium quadridichotomum*, *Platycterium ridleyi*, *Platycterium* sp. ES-2011, *Platycterium stemaria*, *Platycterium superbum*, *Platycterium veitchii*, *Platycterium wallichii*, *Platycterium wandae*, *Platycterium wilhelminae-reginae*, and *Platycterium willinckii*.

[0159] In some embodiments the PtIP-65 polypeptide is derived from a species in the Division Lycophyta.

[0160] In some embodiments the PtIP-65 polypeptide is derived from a species in the Division Lycophyta, Class Isoetopsida or Class Lycopodiopsida.

[0161] In some embodiments the PtIP-65 polypeptide is derived from a species in the Class Isoetopsida Order Se-

laginales. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Isoetopsida*, Order *Selaginales*, Family *Selaginellaceae*. In some embodiments the PtIP-65 polypeptide is derived from a species in the Genus *Selaginella*. In some embodiments the PtIP-65 polypeptide is derived from a *Selaginella* species selected from but not limited to *Selaginella acanthonota*, *Selaginella apoda*, *Selaginella arbuscula*, *Selaginella arenicola*, *Selaginella arizonica*, *Selaginella armata*, *Selaginella asprella*, *Selaginella biformis*, *Selaginella bigelovii*, *Selaginella braunii*, *Selaginella cinerascens*, *Selaginella cordifolia*, *Selaginella deflexa*, *Selaginella delicatula*, *Selaginella densa*, *Selaginella douglasii*, *Selaginella eatonii*, *Selaginella eclipses*, *Selaginella eremophila*, *Selaginella erythropus*, *Selaginella flabellata*, *Selaginella hansenii*, *Selaginella heterodonta*, *Selaginella kraussiana*, *Selaginella krugii*, *Selaginella laxifolia*, *Selaginella lepidophylla*, *Selaginella leucobryoides*, *Selaginella ludoviciana*, *Selaginella mutica*, *Selaginella oregana*, *Selaginella ovifolia*, *Selaginella pallescens*, *Selaginella peruviana*, *Selaginella pilifera*, *Selaginella plana*, *Selaginella plumosa*, *Selaginella pulcherrima*, *Selaginella rupestris*, *Selaginella rupincola*, *Selaginella scopulorum*, *Selaginella selaginoides*, *Selaginella sibirica*, *Selaginella standleyi*, *Selaginella stellata*, *Selaginella subcaulescens*, *Selaginella substipitata*, *Selaginella tenella*, *Selaginella tortipila*, *Selaginella uliginosa*, *Selaginella umbrosa*, *Selaginella uncinata*, *Selaginella underwoodii*, *Selaginella utahensis*, *Selaginella victoriae*, *Selaginella viridissima*, *Selaginella wallacei*, *Selaginella watsonii*, *Selaginella weatherbiana*, *Selaginella willdenowii*, *Selaginella wrightii* and *Selaginella X neomexicana*.

[0162] In some embodiments the PtIP-65 polypeptide is derived from a species in the Class *Lycopodiopsida*, Order *Lycopodiales*. In some embodiments the PtIP-65 polypeptide is derived from a fern species in the Class *Lycopodiopsida*, Order *Lycopodiales* Family *Lycopodiaceae* or Family *Huperziaceae*. In some embodiments the PtIP-65 polypeptide is derived from a species in the Genus *Austrolycopodium*, *Dendrolycopodium*, *Diphasiastrum*, *Diphasium*, *Huperzia*, *Lateristachys*, *Lycopodiastrum*, *Lycopodiella*, *Lycopodium*, *Palhinhaea*, *Pseudodiphasium*, *Pseudolycopodiella*, *Pseudolycopodium* or *Spinulum*.

[0163] In some embodiments the PtIP-65 polypeptide is derived from a species in the Genus *Lycopodium*. In some embodiments the PtIP-65 polypeptide is derived from a *Lycopodium* species selected from but not limited to *Lycopodium alpinum* L., *Lycopodium annotinum* L., *Lycopodium clavatum* L., *Lycopodium complanatum* L., *Lycopodium dendroideum* Michx., *Lycopodium digitatum*, *Lycopodium xhabereri*, *Lycopodium hickeyi*, *Lycopodium xissleri*, *Lycopodium lagopus*, *Lycopodium obscurum* L., *Lycopodium phlegmaria* L., *Lycopodium sabinifolium*, *Lycopodium sitchense*, *Lycopodium tristachyum*, *Lycopodium venustulum*, *Lycopodium venustulum* var. *venustulum*, *Lycopodium venustulum* var. *verticale*, *Lycopodium volubile* and *Lycopodium xzeileri*.

[0164] In some embodiments the PtIP-65 polypeptide is derived from a species in the Genus *Huperzia*. In some embodiments the PtIP-65 polypeptide is derived from a species selected from but not limited to *Huperzia appressa*, *Huperzia arctica*, *Huperzia attenuata*, *Huperzia australiana*, *Huperzia balansae*, *Huperzia billardierei*, *Huperzia brassii*, *Huperzia campiana*, *Huperzia capellae*, *Huperzia carinata*, *Huperzia* cf. *carinata* ARF000603, *Huperzia* cf. *nummulariifolia* ARF001140, *Huperzia* cf. *phlegmaria* ARF000717, *Huperzia* cf. *phlegmaria* ARF000771, *Huperzia* cf. *phlegmaria* ARF000785, *Huperzia* cf. *phlegmaria* ARF001007, *Huperzia* cf. *phlegmaria* ARF002568, *Huperzia* cf. *phlegmaria* ARF002703, *Huperzia* cf. *phlegmaria* Wikstrom 1998, *Huperzia chinensis*, *Huperzia compacta*, *Huperzia crassa*, *Huperzia crispata*, *Huperzia cryptomeriana*, *Huperzia cumingii*, *Huperzia dacrydioides*, *Huperzia dalhousieana*, *Huperzia dichotoma*, *Huperzia emeiensis*, *Huperzia ericifolia*, *Huperzia eversa*, *Huperzia fargesii*, *Huperzia fordii*, *Huperzia funiformis*, *Huperzia goebellii*, *Huperzia haleakalae*, *Huperzia hamiltonii*, *Huperzia heteroclita*, *Huperzia hippuridea*, *Huperzia hippuris*, *Huperzia holstii*, *Huperzia horizontalis*, *Huperzia hunanensis*, *Huperzia hystrix*, *Huperzia lindenii*, *Huperzia linifolia*, *Huperzia lockyeri*, *Huperzia lucidula*, *Huperzia mingcheensis*, *Huperzia miyoshiana*, *Huperzia nanchuanensis*, *Huperzia nummulariifolia*, *Huperzia obtusifolia*, *Huperzia ophioglossoides*, *Huperzia petiolata*, *Huperzia phlegmaria*, *Huperzia phlegmarioides*, *Huperzia phyllantha*, *Huperzia pinifolia*, *Huperzia polydactyla*, *Huperzia prolifera*, *Huperzia reflexa*, *Huperzia rosenstockiana*, *Huperzia rufescens*, *Huperzia salvinoides*, *Huperzia sarmentosa*, *Huperzia selago*, *Huperzia serrata*, *Huperzia sieboldii*, *Huperzia somae*, *Huperzia squarrosa*, *Huperzia subulata*, *Huperzia sutchueniana*, *Huperzia tauri*, *Huperzia taxifolia*, *Huperzia tenuis*, *Huperzia tetragona*, *Huperzia tetrasticha*, *Huperzia unguiculata*, *Huperzia varia*, *Huperzia verticillata* and *Huperzia wilsonii*.

[0165] In some embodiments a PtIP-65 polypeptide has a calculated molecular weight of between about 25kD and about 50kD, between about 27.5kD and about 47.5kD, or between about 30kD and about 45kD.

[0166] In some embodiments the PtIP-65 polypeptide has a modified physical property. As used herein, the term "physical property" refers to any parameter suitable for describing the physical-chemical characteristics of a protein. As used herein, "physical property of interest" and "property of interest" are used interchangeably to refer to physical properties of proteins that are being investigated and/or modified. Examples of physical properties include, but are not limited to net surface charge and charge distribution on the protein surface, net hydrophobicity and hydrophobic residue distribution on the protein surface, surface charge density, surface hydrophobicity density, total count of surface ionizable groups, surface tension, protein size and its distribution in solution, melting temperature, heat capacity, and second virial coefficient. Examples of physical properties also include, but are not limited to solubility, folding, stability, and digestibility. In some embodiments the PtIP-65 polypeptide has increased digestibility of proteolytic fragments in an insect gut. Models for digestion by simulated simulated gastric fluids are known to one skilled in the art (Fuchs, R.L. and J.D. Astwood.

Food Technology 50: 83-88, 1996; Astwood, J.D., et al Nature Biotechnology 14: 1269-1273, 1996; Fu TJ et al J. Agric Food Chem. 50: 7154-7160, 2002).

[0167] In some embodiments variants include polypeptides that differ in amino acid sequence due to mutagenesis. Variant proteins encompassed by the disclosure are biologically active, that is they continue to possess the desired biological activity (i.e. pesticidal activity) of the native protein. In some embodiment the variant will have at least about 10%, at least about 30%, at least about 50%, at least about 70%, at least about 80% or more of the insecticidal activity of the native protein. In some embodiments, the variants may have improved activity over the native protein.

[0168] Bacterial genes quite often possess multiple methionine initiation codons in proximity to the start of the open reading frame. Often, translation initiation at one or more of these start codons will lead to generation of a functional protein. These start codons can include ATG codons. However, bacteria such as *Bacillus* sp. also recognize the codon GTG as a start codon, and proteins that initiate translation at GTG codons contain a methionine at the first amino acid. On rare occasions, translation in bacterial systems can initiate at a TTG codon, though in this event the TTG encodes a methionine. Furthermore, it is not often determined a priori which of these codons are used naturally in the bacterium. Thus, it is understood that use of one of the alternate methionine codons may also lead to generation of pesticidal proteins. These pesticidal proteins are encompassed in the present disclosure and may be used in the methods of the present disclosure. It will be understood that, when expressed in plants, it will be necessary to alter the alternate start codon to ATG for proper translation.

[0169] In another aspect the PtIP-50 polypeptide or PtIP-65 polypeptide may be expressed as a precursor protein with an intervening sequence that catalyzes multi-step, post translational protein splicing.

[0170] In another aspect the PtIP-50 polypeptide or PtIP-65 polypeptide may be encoded by two separate genes where the intein of the precursor protein comes from the two genes, referred to as a split-intein, and the two portions of the precursor are joined by a peptide bond formation.

[0171] In another aspect the PtIP-50 polypeptide is a circular permuted variant.

[0172] In another aspect the PtIP-65 polypeptide is a circular permuted variant.

[0173] In another aspect fusion proteins are provided that include within its amino acid sequence an amino acid sequence comprising a PtIP-50 polypeptide.

[0174] In another aspect fusion proteins are provided that include within its amino acid sequence an amino acid sequence comprising a PtIP-65 polypeptide.

[0175] Methods for design and construction of fusion proteins (and polynucleotides encoding same) are known to those of skill in the art. Polynucleotides encoding a PtIP-50 polypeptide or PtIP-65 polypeptide may be fused to signal sequences which will direct the localization of the PtIP-50 polypeptide or PtIP-65 polypeptide to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of the PtIP-50 polypeptide or PtIP-65 polypeptide of the embodiments from a prokaryotic or eukaryotic cell. For example, in *E. coli*, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the PtIP-50 polypeptide or PtIP-65 polypeptide may be fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the *peIB* signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the *ompA* signal sequence, the signal sequence of the periplasmic *E. coli* heat-labile enterotoxin B-subunit and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-p series) available from New England Biolabs. In a specific embodiment, the PtIP-50 polypeptide or PtIP-65 polypeptide may be fused to the *peIB* pectate lyase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria (see, US Patent Numbers 5,576,195 and 5,846,818). Plant plastid transit peptide / polypeptide fusions are well known in the art (see, US Patent Number 7,193,133). Apoplast transit peptides such as rice or barley alpha-amylase secretion signal are also well known in the art. The plastid transit peptide is generally fused N-terminal to the polypeptide to be targeted (e.g., the fusion partner). In one embodiment, the fusion protein consists essentially of the plastid transit peptide and the PtIP-50 polypeptide or PtIP-65 polypeptide to be targeted. In another embodiment, the fusion protein comprises the plastid transit peptide and the polypeptide to be targeted. In such embodiments, the plastid transit peptide is preferably at the N-terminus of the fusion protein. However, additional amino acid residues may be N-terminal to the plastid transit peptide providing that the fusion protein is at least partially targeted to a plastid. In a specific embodiment, the plastid transit peptide is in the N-terminal half, N-terminal third or N-terminal quarter of the fusion protein. Most or all of the plastid transit peptide is generally cleaved from the fusion protein upon insertion into the plastid. The position of cleavage may vary slightly between plant species, at different plant developmental stages, as a result of specific intercellular conditions or the particular combination of transit peptide/fusion partner used. In one embodiment, the plastid transit peptide cleavage is homogenous such that the cleavage site is identical in a population of fusion proteins. In another embodiment, the plastid transit peptide is not homogenous, such that the cleavage site varies by 1-10 amino acids in a population of fusion proteins. The plastid transit peptide can be recombinantly fused to a second protein in one of several ways. For example, a restriction endonuclease recognition site can be introduced into the nucleotide sequence of the transit peptide at a position corresponding to its C-terminal end and the

same or a compatible site can be engineered into the nucleotide sequence of the protein to be targeted at its N-terminal end. Care must be taken in designing these sites to ensure that the coding sequences of the transit peptide and the second protein are kept "in frame" to allow the synthesis of the desired fusion protein. In some cases, it may be preferable to remove the initiator methionine codon of the second protein when the new restriction site is introduced. The introduction of restriction endonuclease recognition sites on both parent molecules and their subsequent joining through recombinant DNA techniques may result in the addition of one or more extra amino acids between the transit peptide and the second protein. This generally does not affect targeting activity as long as the transit peptide cleavage site remains accessible and the function of the second protein is not altered by the addition of these extra amino acids at its N-terminus. Alternatively, one skilled in the art can create a precise cleavage site between the transit peptide and the second protein (with or without its initiator methionine) using gene synthesis (Stemmer, et al., (1995) *Gene* 164:49-53) or similar methods. In addition, the transit peptide fusion can intentionally include amino acids downstream of the cleavage site. The amino acids at the N-terminus of the mature protein can affect the ability of the transit peptide to target proteins to plastids and/or the efficiency of cleavage following protein import. This may be dependent on the protein to be targeted. See, e.g., Comai, et al., (1988) *J. Biol. Chem.* 263(29):15104-9.

[0176] In some embodiments fusion proteins are provided comprising a PtIP-50 polypeptide or PtIP-65 polypeptide and an insecticidal polypeptide joined by an amino acid linker. In some embodiments fusion proteins are provided comprising a PtIP-50 polypeptide and a PtIP-65 polypeptide joined by an amino acid linker.

[0177] In some embodiments fusion proteins are provided represented by a formula selected from the group consisting of:

R^1 -L- R^2 , R^2 -L- R^1 , R^1 - R^2 or R^2 - R^1

wherein R^1 is a PtIP-50 polypeptide, R^2 is PtIP-65 polypeptide. The R^1 polypeptide is fused either directly or through a linker (L) segment to the R^2 polypeptide. The term "directly" defines fusions in which the polypeptides are joined without a peptide linker. Thus "L" represents a chemical bond or polypeptide segment to which both R^1 and R^2 are fused in frame, most commonly L is a linear peptide to which R^1 and R^2 are bound by amide bonds linking the carboxy terminus of R^1 to the amino terminus of L and carboxy terminus of L to the amino terminus of R^2 . By "fused in frame" is meant that there is no translation termination or disruption between the reading frames of R^1 and R^2 . The linking group (L) is generally a polypeptide of between 1 and 500 amino acids in length. The linkers joining the two molecules are preferably designed to (1) allow the two molecules to fold and act independently of each other, (2) not have a propensity for developing an ordered secondary structure which could interfere with the functional domains of the two proteins, (3) have minimal hydrophobic or charged characteristic which could interact with the functional protein domains and (4) provide steric separation of R^1 and R^2 such that R^1 and R^2 could interact simultaneously with their corresponding receptors on a single cell. Typically surface amino acids in flexible protein regions include Gly, Asn and Ser. Virtually any permutation of amino acid sequences containing Gly, Asn and Ser would be expected to satisfy the above criteria for a linker sequence. Other neutral amino acids, such as Thr and Ala, may also be used in the linker sequence. Additional amino acids may also be included in the linkers due to the addition of unique restriction sites in the linker sequence to facilitate construction of the fusions.

[0178] In some embodiments the linkers comprise sequences selected from the group of formulas: $(Gly_3Ser)_n$, $(Gly_4Ser)_n$, $(Gly_5Ser)_n$, $(Gly_nSer)_n$ or $(AlaGlySer)_n$ where n is an integer. One example of a highly-flexible linker is the (GlySer)-rich spacer region present within the pIII protein of the filamentous bacteriophages, e.g. bacteriophages M13 or fd (Schaller, et al., 1975). This region provides a long, flexible spacer region between two domains of the pIII surface protein. Also included are linkers in which an endopeptidase recognition sequence is included. Such a cleavage site may be valuable to separate the individual components of the fusion to determine if they are properly folded and active in vitro. Examples of various endopeptidases include, but are not limited to, Plasmin, Enterokinase, Kallikerin, Urokinase, Tissue Plasminogen activator, clostripain, Chymosin, Collagenase, Russell's Viper Venom Protease, Postproline cleavage enzyme, V8 protease, Thrombin and factor Xa. In some embodiments the linker comprises the amino acids EEKKN (SEQ ID NO: 201) from the multi-gene expression vehicle (MGEV), which is cleaved by vacuolar proteases as disclosed in US Patent Application Publication Number US 2007/0277263. In other embodiments, peptide linker segments from the hinge region of heavy chain immunoglobulins IgG, IgA, IgM, IgD or IgE provide an angular relationship between the attached polypeptides. Especially useful are those hinge regions where the cysteines are replaced with serines. Linkers of the present disclosure include sequences derived from murine IgG gamma 2b hinge region in which the cysteines have been changed to serines. The fusion proteins are not limited by the form, size or number of linker sequences employed and the only requirement of the linker is that functionally it does not interfere adversely with the folding and function of the individual molecules of the fusion.

[0179] In another aspect chimeric PtIP-50 polypeptides are provided that are created through joining two or more portions of PtIP-50 genes, which originally encoded separate PtIP-50 proteins to create a chimeric gene. The translation of the chimeric gene results in a single chimeric PtIP-50 polypeptide with regions, motifs or domains derived from each of the original polypeptides. In certain embodiments the chimeric protein comprises portions, motifs or domains of PtIP-50 polypeptides in any combination.

[0180] In another aspect chimeric PtIP-65 polypeptides are provided that are created through joining two or more portions of PtIP-65 genes, which originally encoded separate PtIP-65 proteins to create a chimeric gene. The translation of the chimeric gene results in a single chimeric PtIP-65 polypeptide with regions, motifs or domains derived from each of the original polypeptides. In certain embodiments the chimeric protein comprises portions, motifs or domains of the PtIP-65 polypeptides in any combination.

[0181] It is recognized that DNA sequences may be altered by various methods, and that these alterations may result in DNA sequences encoding proteins with amino acid sequences different than that encoded by the wild-type (or native) pesticidal protein. In some embodiments a PtIP-50 polypeptide or PtIP-65 polypeptide may be altered in various ways including amino acid substitutions, deletions, truncations and insertions of one or more amino acids, including up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145 or more amino acid substitutions, deletions and/or insertions or combinations thereof.

[0182] In some embodiments a PtIP-50 polypeptide or PtIP-65 polypeptide comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more amino acids from the N-terminus of the PtIP-50 polypeptide.

[0183] Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a PtIP-50 polypeptide or PtIP-65 polypeptide can be prepared by mutations in the DNA. This may also be accomplished by one of several forms of mutagenesis and/or in directed evolution. In some aspects, the changes encoded in the amino acid sequence will not substantially affect the function of the protein. Such variants will possess the desired pesticidal activity. However, it is understood that the ability of a PtIP-50 polypeptide or PtIP-65 polypeptide to confer pesticidal activity may be improved by the use of such techniques upon the compositions of this disclosure.

[0184] For example, conservative amino acid substitutions may be made at one or more, PtIP-, nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a PtIP-50 polypeptide or PtIP-65 polypeptide without altering the biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include: amino acids with basic side chains (e.g., lysine, arginine, histidine); acidic side chains (e.g., aspartic acid, glutamic acid); polar, negatively charged residues and their amides (e.g., aspartic acid, asparagine, glutamic acid, glutamine); uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine); small aliphatic, nonpolar or slightly polar residues (e.g., Alanine, serine, threonine, proline, glycine); nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); large aliphatic, nonpolar residues (e.g., methionine, leucine, isoleucine, valine, cysteine); beta-branched side chains (e.g., threonine, valine, isoleucine); aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine); large aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan).

[0185] Amino acid substitutions may be made in nonconserved regions that retain function. In general, such substitutions would not be made for conserved amino acid residues or for amino acid residues residing within a conserved motif, where such residues are essential for protein activity. Examples of residues that are conserved and that may be essential for protein activity include, for example, residues that are identical between all proteins contained in an alignment of similar or related toxins to the sequences of the embodiments (e.g., residues that are identical in an alignment of homologous proteins). Examples of residues that are conserved but that may allow conservative amino acid substitutions and still retain activity include, for example, residues that have only conservative substitutions between all proteins contained in an alignment of similar or related toxins to the sequences of the embodiments (e.g., residues that have only conservative substitutions between all proteins contained in the alignment homologous proteins). However, one of skill in the art would understand that functional variants may have minor conserved or nonconserved alterations in the conserved residues. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff, et al., (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.).

[0186] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, (1982) J Mol Biol. 157(1): 105-32). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, and antigens.

[0187] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e., still obtain a biological functionally equivalent protein. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, *ibid*). These are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9) and arginine (-4.5). In making such changes, the substitution of amino acids whose hydropathic indices are within +2 is preferred, those which are within +1 are particularly preferred, and those within +0.5 are even more particularly preferred.

[0188] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. US Patent Number 4,554,101, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

[0189] As detailed in US Patent Number 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0.+0.1); glutamate (+3.0.+0.1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5.+0.1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

[0190] Alternatively, alterations may be made to the protein sequence of many proteins at the amino or carboxy terminus without substantially affecting activity. This can include insertions, deletions or alterations introduced by modern molecular methods, such as PCR, including PCR amplifications that alter or extend the protein coding sequence by virtue of inclusion of amino acid encoding sequences in the oligonucleotides utilized in the PCR amplification. Alternatively, the protein sequences added can include entire protein-coding sequences, such as those used commonly in the art to generate protein fusions. Such fusion proteins are often used to (1) increase expression of a protein of interest (2) introduce a binding domain, enzymatic activity or epitope to facilitate either protein purification, protein detection or other experimental uses known in the art (3) target secretion or translation of a protein to a subcellular organelle, such as the periplasmic space of Gram-negative bacteria, mitochondria or chloroplasts of plants or the endoplasmic reticulum of eukaryotic cells, the latter of which often results in glycosylation of the protein.

[0191] Variant nucleotide and amino acid sequences of the disclosure also encompass sequences derived from mutagenic and recombinogenic procedures such as DNA shuffling. With such a procedure, one or more different PtIP-50 polypeptide coding regions or one or more different PtIP-65 polypeptide coding regions can be used to create a new PtIP-50 polypeptide or PtIP-65 polypeptide possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between a pesticidal gene and other known pesticidal genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased insecticidal activity. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer, (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751; Stemmer, (1994) Nature 370:389-391; Cramer, et al., (1997) Nature Biotech. 15:436-438; Moore, et al., (1997) J. Mol. Biol. 272:336-347; Zhang, et al., (1997) Proc. Natl. Acad. Sci. USA 94:4504-4509; Cramer, et al., (1998) Nature 391:288-291; and US Patent Numbers 5,605,793 and 5,837,458.

[0192] Domain swapping or shuffling is another mechanism for generating altered PtIP-50 polypeptides or PtIP-65 polypeptides. Domains may be swapped between PtIP-50 polypeptides or between PtIP-65 polypeptides, resulting in hybrid or chimeric toxins with improved insecticidal activity or target spectrum. Methods for generating recombinant proteins and testing them for pesticidal activity are well known in the art (see, for example, Naimov, et al., (2001) Appl. Environ. Microbiol. 67:5328-5330; de Maagd, et al., (1996) Appl. Environ. Microbiol. 62:1537-1543; Ge, et al., (1991) J. Biol. Chem. 266:17954-17958; Schnepf, et al., (1990) J. Biol. Chem. 265:20923-20930; Rang, et al., (1999) Appl. Environ. Microbiol. 65:2918-2925).

[0193] Alignment of PtIP-50 homologs (Figures 10a-10o, 11a-11f, 12a-12j, 13a-13e, 14a-14e, 15a-15e) allowed for identification of residues that are highly conserved among natural homologs in this family. Alignment of PtIP-65 homologs (Figures 3a-3i, 4a-4b, 5a-5c, 6a-6c, 7, 8a-8b) allowed for identification of residues that are highly conserved among natural homologs in this family.

Compositions

[0194] Compositions comprising a PtIP-50 polypeptide and a PtIP-65 polypeptide of the disclosure are also embraced. In some embodiments the composition comprises a PtIP-50 polypeptide and a PtIP-65 polypeptide. In some embodiments the composition comprises a PtIP-50/PtIP-65 fusion protein.

Nucleotide Constructs, Expression Cassettes and Vectors

[0195] The use of the term "nucleotide constructs" herein is not intended to limit the embodiments to nucleotide constructs comprising DNA. Those of ordinary skill in the art will recognize that nucleotide constructs particularly polynucleotides and oligonucleotides composed of ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides may also be employed in the methods disclosed herein. The nucleotide constructs, nucleic acids, and nucleotide sequences of the embodiments additionally encompass all complementary forms of such constructs, molecules, and sequences. Further, the nucleotide constructs, nucleotide molecules, and nucleotide sequences of the embodiments encompass all nucleotide constructs, molecules, and sequences which can be employed in the methods of the embodiments for transforming plants including, but not limited to, those comprised of deoxyribonucleotides, ribonucleotides, and combinations thereof. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules

and synthetic analogues. The nucleotide constructs, nucleic acids, and nucleotide sequences of the embodiments also encompass all forms of nucleotide constructs including, but not limited to, single-stranded forms, double-stranded forms, hairpins, and stem-and-loop structures.

5 **[0196]** A further embodiment relates to a transformed organism such as an organism selected from plant and insect cells, bacteria, yeast, baculovirus, protozoa, nematodes and algae. The transformed organism comprises a DNA molecule of the embodiments, an expression cassette comprising the DNA molecule or a vector comprising the expression cassette, which may be stably incorporated into the genome of the transformed organism.

10 **[0197]** The sequences of the embodiments are provided in DNA constructs for expression in the organism of interest. The construct will include 5' and 3' regulatory sequences operably linked to a sequence of the embodiments. The term "operably linked" as used herein refers to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and where necessary to join two protein coding regions in the same reading frame. The construct may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple DNA
15 constructs.

[0198] Such a DNA construct is provided with a plurality of restriction sites for insertion of the PtIP-50 polypeptide or a PtIP-65 polypeptide gene sequence to be under the transcriptional regulation of the regulatory regions. The DNA construct may additionally contain selectable marker genes.

20 **[0199]** The DNA construct will generally include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region (i.e., a promoter), a DNA sequence of the embodiments, and a transcriptional and translational termination region (i.e., termination region) functional in the organism serving as a host. The transcriptional initiation region (i.e., the promoter) may be native, analogous, foreign or heterologous to the host organism and/or to the sequence of the embodiments. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. The term "foreign" as used herein indicates that the promoter is not found in the native organism into which the promoter is
25 introduced. Where the promoter is "foreign" or "heterologous" to the sequence of the embodiments, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked sequence of the embodiments. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence. Where the promoter is a native or natural sequence, the expression of the operably linked sequence is altered from the wild-type expression, which results in an alteration in phenotype.

30 **[0200]** In some embodiments the DNA construct may also include a transcriptional enhancer sequence. As used herein, the term an "enhancer" refers to a DNA 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. Various enhancers are known in the art including for example, introns with gene expression enhancing properties in plants (US Patent Application Publication Number 2009/0144863, the ubiquitin intron (i.e., the maize ubiquitin intron 1
35 (see, for example, NCBI sequence S94464)), the omega enhancer or the omega prime enhancer (Gallie, et al., (1989) Molecular Biology of RNA ed. Cech (Liss, New York) 237-256 and Gallie, et al., (1987) Gene 60:217-25), the CaMV 35S enhancer (see, e.g., Benfey, et al., (1990) EMBO J. 9:1685-96) and the enhancers of US Patent Number 7,803,992 may also be used. The above list of transcriptional enhancers is not meant to be limiting. Any appropriate transcriptional enhancer can be used in the embodiments.

40 **[0201]** The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host or may be derived from another source (i.e., foreign or heterologous to the promoter, the sequence of interest, the plant host or any combination thereof).

[0202] Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau, et al., (1991) Mol. Gen. Genet. 262:141-144; Proudfoot, (1991) Cell 64:671-674; Sanfacon, et al., (1991) Genes Dev. 5:141-149; Mogen, et al., (1990) Plant
45 Cell 2:1261-1272; Munroe, et al., (1990) Gene 91:151-158; Ballas, et al., (1989) Nucleic Acids Res. 17:7891-7903 and Joshi, et al., (1987) Nucleic Acid Res. 15:9627-9639.

[0203] Where appropriate, a nucleic acid may be optimized for increased expression in the host organism. Thus, where the host organism is a plant, the synthetic nucleic acids can be synthesized using plant-preferred codons for improved
50 expression. See, for example, Campbell and Gowri, (1990) Plant Physiol. 92:1-11 for a discussion of host-preferred codon usage. For example, although nucleic acid sequences of the embodiments may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray et al. (1989) Nucleic Acids Res. 17:477-498). Thus, the maize-preferred codon for a particular amino acid may be derived
55 from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray, et al., *supra*. Methods are available in the art for synthesizing plant-preferred genes. See, for example, US Patent Numbers 5,380,831, and 5,436,391 and Murray, et al., (1989) Nucleic Acids Res. 17:477-498, and Liu H et al. Mol Bio Rep 37:677-684, 2010. A Zea maize codon usage table can be also found at kazusa.or.jp/codon/cgi-bin/show-

EP 3 102 684 B1

codon.cgi?species=4577, which can be accessed using the www prefix. Table 4 shows a maize optimal codon analysis (adapted from Liu H et al. Mol Bio Rep 37:677-684, 2010).

Table 4

Amino Acid	Codon	High Count	RSCU	Low Count	RSCU	Amino Acid	Codon	High Count	RSCU	Low Count	RSCU
Phe	UUU	115	0.04	2,301	1.22	Ala	GCU	629	0.17	3,063	1.59
	UUC*	5,269	1.96	1,485	0.78		GCC*	8,057	2.16	1,136	0.59
Ser	UCU	176	0.13	2,498	1.48		GCA	369	0.1	2,872	1.49
	UCC*	3,489	2.48	1,074	0.63		GCG*	5,835	1.57	630	0.33
	UCA	104	0.07	2,610	1.54	Tyr	UAU	71	0.04	1,632	1.22
	UCG*	1,975	1.4	670	0.4		UAC*	3,841	1.96	1,041	0.78
	AGU	77	0.05	1,788	1.06	His	CAU	131	0.09	1,902	1.36
	AGC*	2,617	1.86	1,514	0.89		CAC*	2,800	1.91	897	0.64
Leu	UUA	10	0.01	1,326	0.79	Cys	UGU	52	0.04	1,233	1.12
	UUG	174	0.09	2,306	1.37		UGC*	2,291	1.96	963	0.88
	CUU	223	0.11	2,396	1.43	Gln	CAA	99	0.05	2,312	1.04
	CUC*	5,979	3.08	1,109	0.66		CAG*	3,557	1.95	2,130	0.96
	CUA	106	0.05	1,280	0.76	Arg	CGU	153	0.12	751	0.74
	CUG*	5,161	2.66	1,646	0.98		CGC*	4,278	3.25	466	0.46
Pro	CCU	427	0.22	1,900	1.47		CGA	92	0.07	659	0.65
	CCC*	3,035	1.59	601	0.47		CGG*	1,793	1.36	631	0.62
	CCA	311	0.16	2,140	1.66		AGA	83	0.06	1,948	1.91
	CCG*	3,846	2.02	513	0.4		AGG*	1,493	1.14	1,652	1.62
Ile	AUU	138	0.09	2,388	1.3	Asn	AAU	131	0.07	3,074	1.26
	AUC*	4,380	2.85	1,353	0.74		AAC*	3,814	1.93	1,807	0.74
	AUA	88	0.06	1,756	0.96	Lys	AAA	130	0.05	3,215	0.98
Thr	ACU	136	0.09	1,990	1.43		AAG*	5,047	1.95	3,340	1.02
	ACC*	3,398	2.25	991	0.71	Asp	GAU	312	0.09	4,217	1.38
	ACA	133	0.09	2,075	1.5		GAC*	6,729	1.91	1,891	0.62
	ACG*	2,378	1.57	495	0.36	Gly	GGU	363	0.13	2,301	1.35
Val	GUU	182	0.07	2,595	1.51		GGC*	7,842	2.91	1,282	0.75
	GUC*	4,584	1.82	1,096	0.64		GGA	397	0.15	2,044	1.19
	GUA	74	0.03	1,325	0.77		GGG*	2,186	0.81	1,215	0.71
	GUG*	5,257	2.08	1,842	1.07	Glu	GAA	193	0.06	4,080	1.1
							GAG*	6,010	1.94	3,307	0.9

Codon usage was compared using Chi squared contingency test to identify optimal codons. Codons that occur significantly more often (P\0.01) are indicated with an asterisk.

[0204] A *Glycine max* codon usage table is shown in Table 5 and can also be found at kazusa.or.jp/codon/cgi-bin/show-codon.cgi?species=3847&aa=1&style=N, which can be accessed using the www prefix.

EP 3 102 684 B1

Table 5

TTT	F	21.2	(10493)	TCT	S	18.4	(9107)
TTC	F	21.2	(10487)	TCC	S	12.9	(6409)
TTA	L	9.2	(4545)	TCA	S	15.6	(7712)
TTG	L	22.9	(11340)	TCG	S	4.8	(2397)
CTT	L	23.9	(11829)	CCT	P	18.9	(9358)
CTC	L	17.1	(8479)	CCC	P	10.1	(5010)
CTA	L	8.5	(4216)	CCA	P	19.1	(9461)
CTG	L	12.7	(6304)	CCG	P	4.7	(2312)
ATT	I	25.1	(12411)	ACT	T	17.1	(8490)
ATC	I	16.3	(8071)	ACC	T	14.3	(7100)
ATA	I	12.9	(6386)	ACA	T	14.9	(7391)
ATG	M	22.7	(11218)	ACG	T	4.3	(2147)
GTT	V	26.1	(12911)	GCT	A	26.7	(13201)
GTC	V	11.9	(5894)	GCC	A	16.2	(8026)
GTA	V	7.7	(3803)	GCA	A	21.4	(10577)
GTG	V	21.4	(10610)	GCG	A	6.3	(3123)
TAT	Y	15.7	(7779)	TGT	C	8.1	(3995)
TAC	Y	14.9	(7367)	TGC	C	8.0	(3980)
TAA	*	0.9	(463)	TGA	*	1.0	(480)
TAG	*	0.5	(263)	TGG	W	13.0	(6412)
CAT	H	14.0	(6930)	CGT	R	6.6	(3291)
CAC	H	11.6	(5759)	CGC	R	6.2	(3093)
CAA	Q	20.5	(10162)	CGA	R	4.1	(2018)
CAG	Q	16.2	(8038)	CGG	R	3.1	(1510)
AAT	N	22.4	(11088)	AGT	S	12.6	(6237)
AAC	N	22.8	(11284)	AGC	S	11.3	(5594)
AAA	K	26.9	(13334)	AGA	R	14.8	(7337)
AAG	K	35.9	(17797)	AGG	R	13.3	(6574)
GAT	D	32.4	(16040)	GGT	G	20.9	(10353)
GAC	D	20.4	(10097)	GGC	G	13.4	(6650)
GAA	E	33.2	(16438)	GGA	G	22.3	(11022)
GAG	E	33.2	(16426)	GGG	G	13.0	(6431)

[0205] In some embodiments the recombinant nucleic acid molecule encoding a PtIP-50 polypeptide or a PtIP-65 polypeptide has maize optimized codons.

[0206] Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other well-characterized sequences that may be deleterious to gene expression. The GC content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. The term "host cell" as used herein refers to a cell which contains a vector and supports the replication and/or expression of the expression vector is intended. Host cells may be prokaryotic cells such as *E. coli* or

eukaryotic cells such as yeast, insect, amphibian or mammalian cells or monocotyledonous or dicotyledonous plant cells. An example of a monocotyledonous host cell is a maize host cell. When possible, the sequence is modified to avoid PtIP- hairpin secondary mRNA structures.

[0207] The expression cassettes may additionally contain 5' leader sequences. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein, et al., (1989) Proc. Natl. Acad. Sci. USA 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Gallie, et al., (1995) Gene 165(2):233-238), MDMV leader (Maize Dwarf Mosaic Virus), human immunoglobulin heavy-chain binding protein (BiP) (Macejak, et al., (1991) Nature 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling, et al., (1987) Nature 325:622-625); tobacco mosaic virus leader (TMV) (Gallie, et al., (1989) in Molecular Biology of RNA, ed. Cech (Liss, New York), pp. 237-256) and maize chlorotic mottle virus leader (MCMV) (Lommel, et al., (1991) Virology 81:382-385). See also, Della-Cioppa, et al., (1987) Plant Physiol. 84:965-968. Such constructs may also contain a "signal sequence" or "leader sequence" to facilitate co-translational or post-translational transport of the peptide to certain intracellular structures such as the chloroplast (or other plastid), endoplasmic reticulum or Golgi apparatus.

[0208] "Signal sequence" as used herein refers to a sequence that is known or suspected to result in cotranslational or post-translational peptide transport across the cell membrane. In eukaryotes, this typically involves secretion into the Golgi apparatus, with some resulting glycosylation. Insecticidal toxins of bacteria are often synthesized as protoxins, which are prototypically activated in the gut of the target pest (Chang, (1987) Methods Enzymol. 153:507-516). In some embodiments, the signal sequence is located in the native sequence or may be derived from a sequence of the embodiments. "Leader sequence" as used herein refers to any sequence that when translated, results in an amino acid sequence sufficient to trigger co-translational transport of the peptide chain to a subcellular organelle. Thus, this includes leader sequences targeting transport and/or glycosylation by passage into the endoplasmic reticulum, passage to vacuoles, plastids including chloroplasts, and mitochondria. Nuclear-encoded proteins targeted to the chloroplast thylakoid lumen compartment have a characteristic bipartite transit peptide, composed of a stromal targeting signal peptide and a lumen targeting signal peptide. The stromal targeting information is in the amino-proximal portion of the transit peptide. The lumen targeting signal peptide is in the carboxyl-proximal portion of the transit peptide, and contains all the information for targeting to the lumen. Recent research in proteomics of the higher plant chloroplast has achieved in the identification of numerous nuclear-encoded lumen proteins (Kieselbach et al. FEBS LETT 480:271-276, 2000; Peltier et al. Plant Cell 12:319-341, 2000; Bricker et al. Biochim. Biophys Acta 1503:350-356, 2001), the lumen targeting signal peptide of which can potentially be used in accordance with the present disclosure. About 80 proteins from *Arabidopsis*, as well as homologous proteins from spinach and garden pea, are reported by Kieselbach et al., Photosynthesis Research, 78:249-264, 2003. In particular, Table 2 of this publication discloses 85 proteins from the chloroplast lumen, identified by their accession number (see also US Patent Application Publication 2009/09044298). In addition, the recently published draft version of the rice genome (Goff et al, Science 296:92-100, 2002) is a suitable source for lumen targeting signal peptide which may be used in accordance with the present disclosure.

[0209] Suitable chloroplast transit peptides (CTP) are well known to one skilled in the art also include chimeric CTPs comprising but not limited to, an N-terminal domain, a central domain or a C-terminal domain from a CTP from *Oryza sativa* 1-deoxy-D xylose-5-Phosphate Synthase *Oryza sativa*-Superoxide dismutase *Oryza sativa*-soluble starch synthase *Oryza sativa*-NADP-dependent Malic acid enzyme *Oryza sativa*-Phospho-2-dehydro-3-deoxyheptonate Aldolase 2 *Oryza sativa*-L-Ascorbate peroxidase 5 *Oryza sativa*-Phosphoglucan water dikinase, *Zea Mays* ssRUBISCO, *Zea Mays*-beta-glucosidase, *Zea Mays*-Malate dehydrogenase, *Zea Mays* Thioredoxin M-type US Patent Application Publication 2012/0304336).

[0210] The PtIP-50 polypeptide or a PtIP-65 polypeptide gene to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be synthesized using chloroplast-preferred codons. See, for example, US Patent Number 5,380,831.

[0211] In preparing the expression cassette, the various DNA fragments may be manipulated so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, or removal of restriction sites. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

[0212] A number of promoters can be used in the practice of the embodiments. The promoters can be selected based on the desired outcome. The nucleic acids can be combined with constitutive, tissue-preferred, inducible or other promoters for expression in the host organism. Suitable constitutive promoters for use in a plant host cell include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 1999/43838 and US Patent Number 6,072,050; the core CaMV 35S promoter (Odell, et al., (1985) Nature 313:810-812); rice actin (McElroy, et al., (1990) Plant Cell 2:163-171); ubiquitin (Christensen, et al., (1989) Plant Mol. Biol. 12:619-632 and Christensen, et al., (1992) Plant Mol. Biol. 18:675-689); pEMU (Last, et al., (1991) Theor. Appl. Genet. 81:581-588);

MAS (Velten, et al., (1984) EMBO J. 3:2723-2730); and ALS promoter (US Patent Number 5,659,026). Other constitutive promoters include, for example, those discussed in US Patent Numbers 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142 and 6,177,611.

[0213] Depending on the desired outcome, it may be beneficial to express the gene from an inducible promoter. Of particular interest for regulating the expression of the nucleotide sequences of the embodiments in plants are wound-inducible promoters. Such wound-inducible promoters, may respond to damage caused by insect feeding, and include potato proteinase inhibitor (pin II) gene (Ryan, (1990) Ann. Rev. Phytopath. 28:425-449; Duan, et al., (1996) Nature Biotechnology 14:494-498); *wun1* and *wun2*, US Patent Number 5,428,148; *win1* and *win2* (Stanford, et al., (1989) Mol. Gen. Genet. 215:200-208); *systemin* (McGurl, et al., (1992) Science 225:1570-1573); *WIP1* (Rohmeier, et al., (1993) Plant Mol. Biol. 22:783-792; Eckelkamp, et al., (1993) FEBS Letters 323:73-76); and *MPI* gene (Corderok, et al., (1994) Plant J. 6(2):141-150).

[0214] Additionally, pathogen-inducible promoters may be employed in the methods and nucleotide constructs of the embodiments. Such pathogen-inducible promoters include those from pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen; e.g., PR proteins, SAR proteins, beta-1,3-glucanase, chitinase, etc. See, for example, Redolfi, et al., (1983) Neth. J. Plant Pathol. 89:245-254; Uknes, et al., (1992) Plant Cell 4: 645-656 and Van Loon, (1985) Plant Mol. Virol. 4:111-116. See also, WO 1999/43819.

[0215] Of interest are promoters that are expressed locally at or near the site of pathogen infection. See, for example, Marineau, et al., (1987) Plant Mol. Biol. 9:335-342; Matton, et al., (1989) Molecular Plant-Microbe Interactions 2:325-331; Somsisch, et al., (1986) Proc. Natl. Acad. Sci. USA 83:2427-2430; Somsisch, et al., (1988) Mol. Gen. Genet. 2:93-98 and Yang, (1996) Proc. Natl. Acad. Sci. USA 93:14972-14977. See also, Chen, et al., (1996) Plant J. 10:955-966; Zhang, et al., (1994) Proc. Natl. Acad. Sci. USA 91:2507-2511; Warner, et al., (1993) Plant J. 3:191-201; Siebertz, et al., (1989) Plant Cell 1:961-968; US Patent Number 5,750,386 (nematode-inducible) and the references cited therein. Of particular interest is the inducible promoter for the maize PRms gene, whose expression is induced by the pathogen *Fusarium moniliforme* (see, for example, Cordero, et al., (1992) Physiol. Mol. Plant Path. 41:189-200).

[0216] Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of the chemical induces gene expression or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize *In2-2* promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-1a promoter, which is activated by salicylic acid. Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena, et al., (1991) Proc. Natl. Acad. Sci. USA 88:10421-10425 and McNellis, et al., (1998) Plant J. 14(2):247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz, et al., (1991) Mol. Gen. Genet. 227:229-237 and US Patent Numbers 5,814,618 and 5,789,156).

[0217] Tissue-preferred promoters can be utilized to target enhanced PtIP-50 polypeptide or a PtIP-65 polypeptide expression within a particular plant tissue. Tissue-preferred promoters include those discussed in Yamamoto, et al., (1997) Plant J. 12(2):255-265; Kawamata, et al., (1997) Plant Cell Physiol. 38(7):792-803; Hansen, et al., (1997) Mol. Gen Genet. 254(3):337-343; Russell, et al., (1997) Transgenic Res. 6(2):157-168; Rinehart, et al., (1996) Plant Physiol. 112(3):1331-1341; Van Camp, et al., (1996) Plant Physiol. 112(2):525-535; Canevascini, et al., (1996) Plant Physiol. 112(2):513-524; Yamamoto, et al., (1994) Plant Cell Physiol. 35(5):773-778; Lam, (1994) Results Probl. Cell Differ. 20:181-196; Orozco, et al., (1993) Plant Mol Biol. 23(6):1129-1138; Matsuoka, et al., (1993) Proc Natl. Acad. Sci. USA 90(20):9586-9590 and Guevara-Garcia, et al., (1993) Plant J. 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

[0218] Leaf-preferred promoters are known in the art. See, for example, Yamamoto, et al., (1997) Plant J. 12(2):255-265; Kwon, et al., (1994) Plant Physiol. 105:357-67; Yamamoto, et al., (1994) Plant Cell Physiol. 35(5):773-778; Gotor, et al., (1993) Plant J. 3:509-18; Orozco, et al., (1993) Plant Mol. Biol. 23(6):1129-1138 and Matsuoka, et al., (1993) Proc. Natl. Acad. Sci. USA 90(20):9586-9590.

[0219] Root-preferred or root-specific promoters are known and can be selected from the many available from the literature or isolated *de novo* from various compatible species. See, for example, Hire, et al., (1992) Plant Mol. Biol. 20(2):207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner, (1991) Plant Cell 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger, et al., (1990) Plant Mol. Biol. 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens*) and Miao, et al., (1991) Plant Cell 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also, Bogusz, et al., (1990) Plant Cell 2(7):633-641, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume *Parasponia andersonii* and the related non-nitrogen-fixing nonlegume *Trema tomentosa* are described. The promoters of these genes were linked to a β -glucuronidase reporter gene and introduced into both the nonlegume *Nicotiana tabacum* and the legume *Lotus*

corniculatus, and in both instances root-specific promoter activity was preserved. Leach and Aoyagi, (1991) describe their analysis of the promoters of the highly expressed *rolC* and *rolD* root-inducing genes of *Agrobacterium rhizogenes* (see, Plant Science (Limerick) 79(1):69-76). They concluded that enhancer and tissue-preferred DNA determinants are dissociated in those promoters. Teeri, *et al.*, (1989) used gene fusion to *lacZ* to show that the *Agrobacterium* T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see, EMBO J. 8(2):343-350). The TR1' gene fused to *nptII* (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster, *et al.*, (1995) Plant Mol. Biol. 29(4):759-772) and *rolB* promoter (Capana, *et al.*, (1994) Plant Mol. Biol. 25(4):681-691). See also, US Patent Numbers 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732 and 5,023,179. *Arabidopsis thaliana* root-preferred regulatory sequences are disclosed in US20130117883. [0220] "Seed-preferred" promoters include both "seed-specific" promoters (those promoters active during seed development such as promoters of seed storage proteins) as well as "seed-germinating" promoters (those promoters active during seed germination). See, Thompson, *et al.*, (1989) BioEssays 10:108. Such seed-preferred promoters include, but are not limited to, *Cim1* (cytokinin-induced message); *cZ19B1* (maize 19 kDa zein); and *milps* (myo-inositol-1-phosphate synthase) (see, US Patent Number 6,225,529). Gamma-zein and *Glb-1* are endosperm-specific promoters. For dicots, seed-specific promoters include, but are not limited to, *Kunitz trypsin inhibitor 3 (KTI3)* (Jofuku and Goldberg, (1989) Plant Cell 1:1079-1093), bean β -phaseolin, napin, β -conglycinin, glycinin 1, soybean lectin, and cruciferin. For monocots, seed-specific promoters include, but are not limited to, maize 15 kDa zein, 22 kDa zein, 27 kDa zein, g-zein, waxy, shrunken 1, shrunken 2, globulin 1, etc. See also, WO 2000/12733, where seed-preferred promoters from *end1* and *end2* genes are disclosed. In dicots, seed specific promoters include but are not limited to seed coat promoter from *Arabidopsis*, pBAN; and the early seed promoters from *Arabidopsis*, p26, p63, and p63tr (US Patent Numbers 7,294,760 and 7,847,153). A promoter that has "preferred" expression in a particular tissue is expressed in that tissue to a greater degree than in at least one other plant tissue. Some tissue-preferred promoters show expression almost exclusively in the particular tissue.

[0221] Where low level expression is desired, weak promoters will be used. Generally, the term "weak promoter" as used herein refers to a promoter that drives expression of a coding sequence at a low level. By low level expression at levels of between about 1/1000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts is intended. Alternatively, it is recognized that the term "weak promoters" also encompasses promoters that drive expression in only a few cells and not in others to give a total low level of expression. Where a promoter drives expression at unacceptably high levels, portions of the promoter sequence can be deleted or modified to decrease expression levels.

[0222] Such weak constitutive promoters include, for example the core promoter of the *Rsyn7* promoter (WO 1999/43838 and US Patent Number 6,072,050), and the core 35S *CaMV* promoter. Other constitutive promoters include, for example, those disclosed in US Patent Numbers 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142 and 6,177,611.

[0223] The above list of promoters is not meant to be limiting. Any appropriate promoter can be used in the embodiments.

[0224] Generally, the expression cassette will comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones and 2,4-dichlorophenoxyacetate (2,4-D). Additional examples of suitable selectable marker genes include, but are not limited to, genes encoding resistance to chloramphenicol (Herrera Estrella, *et al.*, (1983) EMBO J. 2:987-992); methotrexate (Herrera Estrella, *et al.*, (1983) Nature 303:209-213 and Meijer, *et al.*, (1991) Plant Mol. Biol. 16:807-820); streptomycin (Jones, *et al.*, (1987) Mol. Gen. Genet. 210:86-91); spectinomycin (Bretagne-Sagnard, *et al.*, (1996) Transgenic Res. 5:131-137); bleomycin (Hille, *et al.*, (1990) Plant Mol. Biol. 7:171-176); sulfonamide (Guerineau, *et al.*, (1990) Plant Mol. Biol. 15:127-136); bromoxynil (Stalker, *et al.*, (1988) Science 242:419-423); glyphosate (Shaw, *et al.*, (1986) Science 233:478-481 and US Patent Application Serial Numbers 10/004,357 and 10/427,692); phosphinothricin (DeBlock, *et al.*, (1987) EMBO J. 6:2513-2518). See generally, Yarranton, (1992) Curr. Opin. Biotech. 3:506-511; Christopherson, *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89:6314-6318; Yao, *et al.*, (1992) Cell 71:63-72; Reznikoff, (1992) Mol. Microbiol. 6:2419-2422; Barkley, *et al.*, (1980) in The Operon, pp. 177-220; Hu, *et al.*, (1987) Cell 48:555-566; Brown, *et al.*, (1987) Cell 49:603-612; Figge, *et al.*, (1988) Cell 52:713-722; Deuschle, *et al.*, (1989) Proc. Natl. Acad. Sci. USA 86:5400-5404; Fuerst, *et al.*, (1989) Proc. Natl. Acad. Sci. USA 86:2549-2553; Deuschle, *et al.*, (1990) Science 248:480-483; Gossen, (1993) Ph.D. Thesis, University of Heidelberg; Reines, *et al.*, (1993) Proc. Natl. Acad. Sci. USA 90:1917-1921; Labow, *et al.*, (1990) Mol. Cell. Biol. 10:3343-3356; Zambretti, *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89:3952-3956; Baim, *et al.*, (1991) Proc. Natl. Acad. Sci. USA 88:5072-5076; Wyborski, *et al.*, (1991) Nucleic Acids Res. 19:4647-4653; Hillenand-Wissman, (1989) Topics Mol. Struct. Biol. 10:143-162; De-genkolb, *et al.*, (1991) Antimicrob. Agents Chemother. 35:1591-1595; Kleinschmidt, *et al.*, (1988) Biochemistry 27:1094-1104; Bonin, (1993) Ph.D. Thesis, University of Heidelberg; Gossen, *et al.*, (1992) Proc. Natl. Acad. Sci. USA

89:5547-5551; Oliva, et al., (1992) Antimicrob. Agents Chemother. 36:913-919; Hlavka, et al., (1985) Handbook of Experimental Pharmacology, Vol. 78 (Springer-Verlag, Berlin) and Gill, et al., (1988) Nature 334:721-724.

[0225] The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the embodiments.

5

Plant Transformation

[0226] The methods of the embodiments involve introducing a polypeptide or polynucleotide into a plant. "Introducing" is as used herein means presenting to the plant the polynucleotide or polypeptide in such a manner that the sequence gains access to the interior of a cell of the plant. The methods of the embodiments do not depend on a particular method for introducing a polynucleotide or polypeptide into a plant, only that the polynucleotide or polypeptides gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotide or polypeptides into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

10

[0227] "Stable transformation" is as used herein means that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" as used herein means that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant or a polypeptide is introduced into a plant. "Plant" as used herein refers to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells and pollen).

15

[0228] Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway, et al., (1986) Biotechniques 4:320-334), electroporation (Riggs, et al., (1986) Proc. Natl. Acad. Sci. USA 83:5602-5606), *Agrobacterium-mediated* transformation (US Patent Numbers 5,563,055 and 5,981,840), direct gene transfer (Paszowski, et al., (1984) EMBO J. 3:2717-2722) and ballistic particle acceleration (see, for example, US Patent Numbers 4,945,050; 5,879,918; 5,886,244 and 5,932,782; Tomes, et al., (1995) in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg and Phillips, (Springer-Verlag, Berlin) and McCabe, et al., (1988) Biotechnology 6:923-926) and Lecl transformation (WO 00/28058). For potato transformation see, Tu, et al., (1998) Plant Molecular Biology 37:829-838 and Chong, et al., (2000) Transgenic Research 9:71-78. Additional transformation procedures can be found in Weissinger, et al., (1988) Ann. Rev. Genet. 22:421-477; Sanford, et al., (1987) Particulate Science and Technology 5:27-37 (onion); Christou, et al., (1988) Plant Physiol. 87:671-674 (soybean); McCabe, et al., (1988) Bio/Technology 6:923-926 (soybean); Finer and McMullen, (1991) In Vitro Cell Dev. Biol. 27P:175-182 (soybean); Singh, et al., (1998) Theor. Appl. Genet. 96:319-324 (soybean); Datta, et al., (1990) Biotechnology 8:736-740 (rice); Klein, et al., (1988) Proc. Natl. Acad. Sci. USA 85:4305-4309 (maize); Klein, et al., (1988) Biotechnology 6:559-563 (maize); US Patent Numbers 5,240,855; 5,322,783 and 5,324,646; Klein, et al., (1988) Plant Physiol. 91:440-444 (maize); Fromm, et al., (1990) Biotechnology 8:833-839 (maize); Hooykaas-Van Slogteren, et al., (1984) Nature (London) 311:763-764; US Patent Number 5,736,369 (cereals); Bytebier, et al., (1987) Proc. Natl. Acad. Sci. USA 84:5345-5349 (Liliaceae); De Wet, et al., (1985) in The Experimental Manipulation of Ovule Tissues, ed. Chapman, et al., (Longman, New York), pp. 197-209 (pollen); Kaeppeler, et al., (1990) Plant Cell Reports 9:415-418 and Kaeppeler, et al., (1992) Theor. Appl. Genet. 84:560-566 (whisker-mediated transformation); D'Halluin, et al., (1992) Plant Cell 4:1495-1505 (electroporation); Li, et al., (1993) Plant Cell Reports 12:250-255 and Christou and Ford, (1995) Annals of Botany 75:407-413 (rice); Osjoda, et al., (1996) Nature Biotechnology 14:745-750 (maize via *Agrobacterium tumefaciens*).

20

25

30

35

40

[0229] In specific embodiments, the sequences of the embodiments can be provided to a plant using a variety of transient transformation methods. Such transient transformation methods include, but are not limited to, the introduction of the PtIP-50 polypeptide or a PtIP-65 polypeptide or variants and fragments thereof directly into the plant or the introduction of the PtIP-50 polypeptide or a PtIP-65 polypeptide transcript into the plant. Such methods include, for example, microinjection or particle bombardment. See, for example, Crossway, et al., (1986) Mol Gen. Genet. 202:179-185; Nomura, et al., (1986) Plant Sci. 44:53-58; Hepler, et al., (1994) Proc. Natl. Acad. Sci. 91:2176-2180 and Hush, et al., (1994) The Journal of Cell Science 107:775-784. Alternatively, the PtIP-50 polypeptide and/or the PtIP-65 polypeptide polynucleotide can be transiently transformed into the plant using techniques known in the art. Such techniques include viral vector system and the precipitation of the polynucleotide in a manner that precludes subsequent release of the DNA. Thus, transcription from the particle-bound DNA can occur, but the frequency with which it is released to become integrated into the genome is greatly reduced. Such methods include the use of particles coated with polyethylimine (PEI; Sigma #P3143).

45

50

55

[0230] Methods are known in the art for the targeted insertion of a polynucleotide at a specific location in the plant genome. In one embodiment, the insertion of the polynucleotide at a desired genomic location is achieved using a site-specific recombination system. See, for example, WO 1999/25821, WO 1999/25854, WO 1999/25840, WO 1999/25855

and WO 1999/25853. Briefly, the polynucleotide of the embodiments can be contained in transfer cassette flanked by two non-identical recombination sites. The transfer cassette is introduced into a plant have stably incorporated into its genome a target site which is flanked by two non-identical recombination sites that correspond to the sites of the transfer cassette. An appropriate recombinase is provided and the transfer cassette is integrated at the target site. The polynucleotide of interest is thereby integrated at a specific chromosomal position in the plant genome.

[0231] Plant transformation vectors may be comprised of one or more DNA vectors needed for achieving plant transformation. For example, it is a common practice in the art to utilize plant transformation vectors that are comprised of more than one contiguous DNA segment. These vectors are often referred to in the art as "binary vectors". Binary vectors as well as vectors with helper plasmids are most often used for *Agrobacterium*-mediated transformation, where the size and complexity of DNA segments needed to achieve efficient transformation is quite large, and it is advantageous to separate functions onto separate DNA molecules. Binary vectors typically contain a plasmid vector that contains the cis-acting sequences required for T-DNA transfer (such as left border and right border), a selectable marker that is engineered to be capable of expression in a plant cell, and a "gene of interest" (a gene engineered to be capable of expression in a plant cell for which generation of transgenic plants is desired). Also present on this plasmid vector are sequences required for bacterial replication. The cis-acting sequences are arranged in a fashion to allow efficient transfer into plant cells and expression therein. For example, the selectable marker gene and the pesticidal gene are located between the left and right borders. Often a second plasmid vector contains the trans-acting factors that mediate T-DNA transfer from *Agrobacterium* to plant cells. This plasmid often contains the virulence functions (Vir genes) that allow infection of plant cells by *Agrobacterium*, and transfer of DNA by cleavage at border sequences and vir-mediated DNA transfer, as is understood in the art (Hellens and Mullineaux, (2000) Trends in Plant Science 5:446-451). Several types of *Agrobacterium* strains (e.g. LBA4404, GV3101, EHA101, EHA105, etc.) can be used for plant transformation. The second plasmid vector is not necessary for transforming the plants by other methods such as microprojection, microinjection, electroporation, polyethylene glycol, etc.

[0232] In general, plant transformation methods involve transferring heterologous DNA into target plant cells (e.g., immature or mature embryos, suspension cultures, undifferentiated callus, protoplasts, etc.), followed by applying a maximum threshold level of appropriate selection (depending on the selectable marker gene) to recover the transformed plant cells from a group of untransformed cell mass. Following integration of heterologous foreign DNA into plant cells, one then applies a maximum threshold level of appropriate selection in the medium to kill the untransformed cells and separate and proliferate the putatively transformed cells that survive from this selection treatment by transferring regularly to a fresh medium. By continuous passage and challenge with appropriate selection, one identifies and proliferates the cells that are transformed with the plasmid vector. Molecular and biochemical methods can then be used to confirm the presence of the integrated heterologous gene of interest into the genome of the transgenic plant.

[0233] Explants are typically transferred to a fresh supply of the same medium and cultured routinely. Subsequently, the transformed cells are differentiated into shoots after placing on regeneration medium supplemented with a maximum threshold level of selecting agent. The shoots are then transferred to a selective rooting medium for recovering rooted shoot or plantlet. The transgenic plantlet then grows into a mature plant and produces fertile seeds (e.g., Hiei, et al., (1994) The Plant Journal 6:271-282; Ishida, et al., (1996) Nature Biotechnology 14:745-750). Explants are typically transferred to a fresh supply of the same medium and cultured routinely. A general description of the techniques and methods for generating transgenic plants are found in Ayres and Park, (1994) Critical Reviews in Plant Science 13:219-239 and Bommineni and Jauhar, (1997) Maydica 42:107-120. Since the transformed material contains many cells; both transformed and non-transformed cells are present in any piece of subjected target callus or tissue or group of cells. The ability to kill non-transformed cells and allow transformed cells to proliferate results in transformed plant cultures. Often, the ability to remove non-transformed cells is a limitation to rapid recovery of transformed plant cells and successful generation of transgenic plants.

[0234] The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick, et al., (1986) Plant Cell Reports 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive or inducible expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure that expression of the desired phenotypic characteristic has been achieved.

[0235] The nucleotide sequences of the embodiments may be provided to the plant by contacting the plant with a virus or viral nucleic acids. Generally, such methods involve incorporating the nucleotide construct of interest within a viral DNA or RNA molecule. It is recognized that the recombinant proteins of the embodiments may be initially synthesized as part of a viral polyprotein, which later may be processed by proteolysis *in vivo* or *in vitro* to produce the desired PtIP-50 polypeptide or a PtIP-65 polypeptide. It is also recognized that such a viral polyprotein, comprising at least a portion of the amino acid sequence of a PtIP-50 polypeptide or a PtIP-65 polypeptide of the embodiments, may have the desired pesticidal activity. Such viral polyproteins and the nucleotide sequences that encode for them are encompassed by the embodiments. Methods for providing plants with nucleotide constructs and producing the encoded proteins in the plants,

which involve viral DNA or RNA molecules are known in the art. See, for example, US Patent Numbers 5,889,191; 5,889,190; 5,866,785; 5,589,367 and 5,316,931.

[0236] Methods for transformation of chloroplasts are known in the art. See, for example, Svab, et al., (1990) Proc. Natl. Acad. Sci. USA 87:8526-8530; Svab and Maliga, (1993) Proc. Natl. Acad. Sci. USA 90:913-917; Svab and Maliga, (1993) EMBO J. 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-borne transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride, et al., (1994) Proc. Natl. Acad. Sci. USA 91:7301-7305.

[0237] The embodiments further relate to plant-propagating material of a transformed plant of the embodiments including, but not limited to, seeds, tubers, corms, bulbs, leaves and cuttings of roots and shoots.

[0238] The embodiments may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables ornamentals, and conifers.

[0239] Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum. Conifers that may be employed in practicing the embodiments include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). Plants of the embodiments include crop plants (for example, corn, alfalfa, sunflower, *Brassica*, soybean, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, etc.), such as corn and soybean plants.

[0240] Turf grasses include, but are not limited to: annual bluegrass (*Poa annua*); annual ryegrass (*Lolium multiflorum*); Canada bluegrass (*Poa compressa*); Chewing's fescue (*Festuca rubra*); colonial bentgrass (*Agrostis tenuis*); creeping bentgrass (*Agrostis palustris*); crested wheatgrass (*Agropyron desertorum*); fairway wheatgrass (*Agropyron cristatum*); hard fescue (*Festuca longifolia*); Kentucky bluegrass (*Poa pratensis*); orchardgrass (*Dactylis glomerata*); perennial ryegrass (*Lolium perenne*); red fescue (*Festuca rubra*); redbud (*Agrostis alba*); rough bluegrass (*Poa trivialis*); sheep fescue (*Festuca ovina*); smooth bromegrass (*Bromus inermis*); tall fescue (*Festuca arundinacea*); timothy (*Phleum pratense*); velvet bentgrass (*Agrostis canina*); weeping alkaligrass (*Puccinellia distans*); western wheatgrass (*Agropyron smithii*); Bermuda grass (*Cynodon* spp.); St. Augustine grass (*Stenotaphrum secundatum*); zoysia grass (*Zoysia* spp.); Bahia grass (*Paspalum notatum*); carpet grass (*Axonopus affinis*); centipede grass (*Eremochloa ophiuroides*); kikuyu grass (*Pennisetum clandestinum*); seashore paspalum (*Paspalum vaginatum*); blue gramma (*Bouteloua gracilis*); buffalo grass (*Buchloe dactyloids*); sideoats gramma (*Bouteloua curtipendula*).

[0241] Plants of interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, millet, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, flax, castor, olive, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

Evaluation of Plant Transformation

[0242] Following introduction of heterologous foreign DNA into plant cells, the transformation or integration of heterologous gene in the plant genome is confirmed by various methods such as analysis of nucleic acids, proteins and metabolites associated with the integrated gene.

[0243] PCR analysis is a rapid method to screen transformed cells, tissue or shoots for the presence of incorporated gene at the earlier stage before transplanting into the soil (Sambrook and Russell, (2001) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). PCR is carried out using oligonucleotide primers specific to the gene of interest or *Agrobacterium* vector background, etc.

[0244] Plant transformation may be confirmed by Southern blot analysis of genomic DNA (Sambrook and Russell, (2001) *supra*). In general, total DNA is extracted from the transformant, digested with appropriate restriction enzymes, fractionated in an agarose gel and transferred to a nitrocellulose or nylon membrane. The membrane or "blot" is then probed with, for example, radiolabeled ³²P target DNA fragment to confirm the integration of introduced gene into the plant genome according to standard techniques (Sambrook and Russell, (2001) *supra*).

[0245] In Northern blot analysis, RNA is isolated from specific tissues of transformant, fractionated in a formaldehyde agarose gel, and blotted onto a nylon filter according to standard procedures that are routinely used in the art (Sambrook and Russell, (2001) *supra*). Expression of RNA encoded by the pesticidal gene is then tested by hybridizing the filter to a radioactive probe derived from a pesticidal gene, by methods known in the art (Sambrook and Russell, (2001) *supra*).

[0246] Western blot and biochemical assays may be carried out on the transgenic plants to confirm the presence of protein encoded by the pesticidal gene by standard procedures (Sambrook and Russell, 2001, *supra*) using antibodies that bind to one or more epitopes present on the PtIP-50 polypeptide or the PtIP-65 polypeptide.

Stacking of traits in transgenic plant

[0247] Transgenic plants may comprise a stack of one or more insecticidal polynucleotides disclosed herein with one or more additional polynucleotides resulting in the production or suppression of multiple polypeptide sequences. Transgenic plants comprising stacks of polynucleotide sequences can be obtained by either or both of traditional breeding methods or through genetic engineering methods. These methods include, but are not limited to, breeding individual lines each comprising a polynucleotide of interest, transforming a transgenic plant comprising a gene disclosed herein with a subsequent gene and co-transformation of genes into a single plant cell. As used herein, the term "stacked" includes having the multiple traits present in the same plant (i.e., both traits are incorporated into the nuclear genome, one trait is incorporated into the nuclear genome and one trait is incorporated into the genome of a plastid or both traits are incorporated into the genome of a plastid). In one non-limiting example, "stacked traits" comprise a molecular stack where the sequences are physically adjacent to each other. A trait, as used herein, refers to the phenotype derived from a particular sequence or groups of sequences. Co-transformation of genes can be carried out using single transformation vectors comprising multiple genes or genes carried separately on multiple vectors. If the sequences are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be combined at any time and in any order. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (*trans*) or contained on the same transformation cassette (*cis*). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the polynucleotide of interest. This may be combined with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO 1999/25821, WO 1999/25854, WO 1999/25840, WO 1999/25855 and WO 1999/25853.

[0248] In some embodiments the polynucleotides encoding the PtIP-50 polypeptide or the PtIP-65 polypeptide disclosed herein, alone or stacked with one or more additional insect resistance traits can be stacked with one or more additional input traits (e.g., herbicide resistance, fungal resistance, virus resistance, stress tolerance, disease resistance, male sterility, and stalk strength) or output traits (e.g., increased yield, modified starches, improved oil profile, balanced amino acids, high lysine or methionine, increased digestibility, improved fiber quality, and drought resistance). Thus, the polynucleotide embodiments can be used to provide a complete agronomic package of improved crop quality with the ability to flexibly and cost effectively control any number of agronomic pests.

[0249] Other events with regulatory approval are well known to one skilled in the art and can be found at the Center for Environmental Risk Assessment (cera-gmc.org/?action=gm_crop_database, which can be accessed using the www prefix) and at the International Service for the Acquisition of Agri-Biotech Applications (isaaa.org/gmapprovaldatabase/default.asp, which can be accessed using the www prefix).

Gene silencing

[0250] In some embodiments the stacked trait may be in the form of silencing of one or more polynucleotides of interest resulting in suppression of one or more target pest polypeptides. In some embodiments the silencing is achieved through the use of a suppression DNA construct.

Use in Pesticidal Control

[0251] General methods for employing strains comprising a nucleic acid sequence of the embodiments or a variant thereof, in pesticide control or in engineering other organisms as pesticidal agents are known in the art. See, for example

US Patent Number 5,039,523 and EP 0480762A2.

[0252] Microorganism hosts that are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest may be selected. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the PtIP-50 polypeptide or the PtIP-65 polypeptide, and desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

[0253] Such microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms such as bacteria, e.g., *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylius*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*, fungi, particularly yeast, e.g., *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacterium*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes entrophus*, *Clavibacter xyli* and *Azotobacter vinelandii* and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces roseus*, *S. odorus*, *Kluyveromyces veronae*, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms. Host organisms of particular interest include yeast, such as *Rhodotorula spp.*, *Aureobasidium spp.*, *Saccharomyces spp.* (such as *S. cerevisiae*), *Sporobolomyces spp.*, phylloplane organisms such as *Pseudomonas spp.* (such as *P. aeruginosa*, *P. fluorescens*, *P. chlororaphis*), *Erwinia spp.*, and *Flavobacterium spp.*, and other such organisms, including *Agrobacterium tumefaciens*, *E. coli*, *Bacillus subtilis*, and *Bacillus cereus*.

[0254] Genes encoding the PtIP-50 polypeptide and/or the PtIP-65 polypeptide of the embodiments can be introduced into microorganisms that multiply on plants (epiphytes) to deliver PtIP-50 polypeptide and/or the PtIP-65 polypeptides to potential target pests. Epiphytes, for example, can be gram-positive or gram-negative bacteria.

[0255] Root-colonizing bacteria, for example, can be isolated from the plant of interest by methods known in the art. Specifically, a *Bacillus cereus* strain that colonizes roots can be isolated from roots of a plant (see, for example, Handelsman et al. (1991) Appl. Environ. Microbiol. 56:713-718). Genes encoding the PtIP-50 polypeptide and/or the PtIP-65 polypeptide of the embodiments can be introduced into a root-colonizing *Bacillus cereus* by standard methods known in the art.

[0256] Genes encoding PtIP-50 polypeptides and/or the PtIP-65 polypeptides can be introduced, for example, into the root-colonizing *Bacillus* by means of electro transformation. Specifically, genes encoding the PtIP-50 polypeptides and/or the PtIP-65 polypeptides can be cloned into a shuttle vector, for example, pHT3101 (Lerecius, et al., (1989) FEMS Microbiol. Letts. 60:211-218). The shuttle vector pHT3101 containing the coding sequence for the particular PtIP-50 polypeptide and/or the PtIP-65 polypeptide gene can, for example, be transformed into the root-colonizing *Bacillus* by means of electroporation (Lerecius, et al., (1989) FEMS Microbiol. Letts. 60:211-218).

[0257] Expression systems can be designed so that PtIP-50 polypeptides and/or the PtIP-65 polypeptides are secreted outside the cytoplasm of gram-negative bacteria, such as *E. coli*, for example. Advantages of having a PtIP-50 polypeptide and/or a PtIP-65 polypeptide secreted are: (1) avoidance of potential cytotoxic effects of the PtIP-50 polypeptide and/or the PtIP-65 polypeptide expressed; and (2) improvement in the efficiency of purification of the PtIP-50 polypeptide and/or the PtIP-65 polypeptide, including, but not limited to, increased efficiency in the recovery and purification of the protein per volume cell broth and decreased time and/or costs of recovery and purification per unit protein.

[0258] PtIP-50 polypeptides and/or the PtIP-65 polypeptides can be made to be secreted in *E. coli*, for example, by fusing an appropriate *E. coli* signal peptide to the amino-terminal end of the PtIP-50 polypeptide and/or the PtIP-65 polypeptide. Signal peptides recognized by *E. coli* can be found in proteins already known to be secreted in *E. coli*, for example the OmpA protein (Ghrayeb, et al., (1984) EMBO J, 3:2437-2442). OmpA is a major protein of the *E. coli* outer membrane, and thus its signal peptide is thought to be efficient in the translocation process. Also, the OmpA signal peptide does not need to be modified before processing as may be the case for other signal peptides, for example lipoprotein signal peptide (Duffaud, et al., (1987) Meth. Enzymol. 153:492).

[0259] PtIP-50 polypeptides and/or the PtIP-65 polypeptides of the embodiments can be fermented in a bacterial host and the resulting bacteria processed and used as a microbial spray in the same manner that *Bt* strains have been used as insecticidal sprays. In the case of a PtIP-50 polypeptide(s) and/or the PtIP-65 polypeptide(s) that is secreted from *Bacillus*, the secretion signal is removed or mutated using procedures known in the art. Such mutations and/or deletions prevent secretion of the PtIP-50 polypeptide(s) and/or the PtIP-65 polypeptide(s) into the growth medium during the fermentation process. The PtIP-50 polypeptide and/or the PtIP-65 polypeptide are retained within the cell, and the cells are then processed to yield the encapsulated PtIP-50 polypeptide and/or the PtIP-65 polypeptide. Any suitable micro-

organism can be used for this purpose. *Pseudomonas* has been used to express *Bt* toxins as encapsulated proteins and the resulting cells processed and sprayed as an insecticide (Gaertner, et al., (1993), in: Advanced Engineered Pesticides, ed. Kim).

5 [0260] Alternatively, the PtIP-50 polypeptide and/or the PtIP-65 polypeptide are produced by introducing a heterologous gene into a cellular host. Expression of the heterologous gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. These cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated PtIP-50 polypeptide and/or the PtIP-65 polypeptide may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, 10 and foliage of plants. See, for example EPA 0192319, and the references cited therein.

Pesticidal Compositions

15 [0261] In some embodiments the active ingredients can be applied in the form of compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with other compounds. These compounds can be fertilizers, weed killers, Cryoprotectants, surfactants, detergents, pesticidal soaps, dormant oils, polymers, and/or time-release or biodegradable carrier formulations that permit long-term dosing of a target area following a single application of the formulation. They can also be selective herbicides, chemical insecticides, virucides, microbicides, amoebicides, pesticides, fungicides, bacteriocides, nematocides, molluscicides or mixtures of several of these preparations, if desired, 20 together with further agriculturally acceptable carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers. Likewise the formulations may be prepared into edible "baits" or fashioned into pest "traps" to permit feeding or ingestion by a target pest of the pesticidal formulation.

25 [0262] Methods of applying an active ingredient or an agrochemical composition that contains at least one of the PtIP-50 polypeptide and/or the PtIP-65 polypeptide produced by the bacterial strains include leaf application, seed coating and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

30 [0263] The composition may be formulated as a powder, dust, pellet, granule, spray, emulsion, colloid, solution or such like, and may be prepared by such conventional means as desiccation, lyophilization, homogenation, extraction, filtration, centrifugation, sedimentation or concentration of a culture of cells comprising the polypeptide. In all such compositions that contain at least one such pesticidal polypeptide, the polypeptide may be present in a concentration of from about 1% to about 99% by weight.

35 [0264] Lepidopteran, Dipteran, Heteropteran, nematode, Hemiptera or Coleopteran pests may be killed or reduced in numbers in a given area by the methods of the disclosure or may be prophylactically applied to an environmental area to prevent infestation by a susceptible pest. Preferably the pest ingests or is contacted with, a pesticidally-effective amount of the polypeptide. "Pesticidally-effective amount" as used herein refers to an amount of the pesticide that is able to bring about death to at least one pest or to noticeably reduce pest growth, feeding or normal physiological development. This amount will vary depending on such factors as, for example, the specific target pests to be controlled, 40 the specific environment, location, plant, crop or agricultural site to be treated, the environmental conditions and the method, rate, concentration, stability, and quantity of application of the pesticidally-effective polypeptide composition. The formulations may also vary with respect to climatic conditions, environmental considerations, and/or frequency of application and/or severity of pest infestation.

45 [0265] The pesticide compositions described may be made by formulating either the bacterial cell, Crystal and/or spore suspension or isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, desiccated or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The formulated compositions may be in the form of a dust or granular material or a suspension in oil (vegetable or mineral) or water or oil/water emulsions or as a wetttable powder or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers 50 can be solid or liquid and are well known in the art. The term "agriculturally-acceptable carrier" covers all adjuvants, inert components, dispersants, surfactants, tackifiers, binders, etc. that are ordinarily used in pesticide formulation technology; these are well known to those skilled in pesticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, e.g., by homogeneously mixing, blending and/or grinding the pesticidal composition with suitable adjuvants using conventional formulation techniques. Suitable formulations and application methods are described in US Patent Number 6,468,523. The plants can also be treated with one or more chemical compositions, including one or more herbicide, insecticides or fungicides. Exemplary chemical compositions include: Fruits/Vegetables Herbicides: Atrazine, Bromacil, Diuron, Glyphosate, Linuron, Metribuzin, Simazine, Trifluralin, Fluazifop, Glufosinate, Halo sulfuron Gowan, Paraquat, Propyzamide, Sethoxydim, Butafenacil, Halosulfuron, Indaziflam; 55

Fruits/Vegetables Insecticides: Aldicarb, *Bacillus thuriangiensis*, Carbaryl, Carbofuran, Chlorpyrifos, Cypermethrin, Deltamethrin, Diazinon, Malathion, Abamectin, Cyfluthrin/beta-cyfluthrin, Esfenvalerate, Lambda-cyhalothrin, Acequinocyl, Bifenazate, Methoxyfenozide, Novaluron, Chromafenozide, Thiacloprid, Dinotefuran, FluaCrypyrim, Tolfenpyrad, Clothianidin, Spirodiclofen, Gamma-cyhalothrin, Spiromesifen, Spinosad, Rynaxypyr, Cyazypyr, Spinoteram, Triflumuron, Spirotetramat, Imidacloprid, Flubendiamide, Thiodicarb, Metaflumizone, Sulfoxaflor, Cyflumetofen, Cyanopyrafen, Imidacloprid, Clothianidin, Thiamethoxam, Spinotoram, Thiodicarb, Flonicamid, Methiocarb, Emamectin-benzoate, Indoxacarb, Forthiazate, Fenamiphos, Cadusaphos, Pyriproxifen, Fenbutatin-oxid, Hexthiazox, Methomyl, 4-[[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on]; Fruits/Vegetables Fungicides: Carbendazim, Chlorothalonil, EBDs, Sulphur, Thiophanate-methyl, Azoxystrobin, Cymoxanil, Fluazinam, Fosetyl, Iprodione, Kresoxim-methyl, Metalaxyl/mefenoxam, Trifloxystrobin, Ethaboxam, Iprovalicarb, Trifloxystrobin, Fenhexamid, Oxpoconazole fumarate, Cyazofamid, Fenamidone, Zoxamide, Picoxystrobin, Pyraclostrobin, Cyflufenamid, Boscalid; Cereals Herbicides: Isoproturon, Bromoxynil, Ioxynil, Phenoxies, Chlorsulfuron, Clodinafop, Diclofop, Diflufenican, Fenoxaprop, Florasulam, Fluoroxypyr, Metsulfuron, Triasulfuron, Flucarbazone, Iodosulfuron, Propoxycarbazone, Picolinafen, Mesosulfuron, Befluthamid, Pinoxaden, Amidosulfuron, Thifensulfuron Methyl, Tribenuron, Flupyrsulfuron, Sulfosulfuron, Pyrasulfotole, Pyroxosulam, Flufenacet, Tralkoxydim, Pyroxasulfon; Cereals Fungicides: Carbendazim, Chlorothalonil, Azoxystrobin, Cyproconazole, Cyprodinil, Fenpropimorph, Epoxiconazole, Kresoxim-methyl, Quinoxifen, Tebuconazole, Trifloxystrobin, Simeconazole, Picoxystrobin, Pyraclostrobin, Dimoxystrobin, Prothioconazole, Fluoxastrobin; Cereals Insecticides: Dimethoate, Lambda-cyhalothrin, Deltamethrin, alpha-Cypermethrin, beta-cyfluthrin, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinotefuran, Chlorpyrifos, Metamidophos, Oxidemethon-methyl, Pirimicarb, Methiocarb; Maize Herbicides: Atrazine, Alachlor, Bromoxynil, Acetochlor, Dicamba, Clopyralid, (S-) Dimethenamid, Glufosinate, Glyphosate, Isoxaflutole, (S-)Metolachlor, Mesotrione, Nicosulfuron, Primisulfuron, Rimsulfuron, Sulcotrione, Foramsulfuron, Topramezone, Tembotrione, Saflufenacil, Thiencarbazone, Flufenacet, Pyroxasulfon; Maize Insecticides: Carbofuran, Chlorpyrifos, Bifenthrin, Fipronil, Imidacloprid, Lambda-Cyhalothrin, Tefluthrin, Terbufos, Thiamethoxam, Clothianidin, Spiromesifen, Flubendiamide, Triflumuron, Rynaxypyr, Deltamethrin, Thiodicarb, beta-Cyfluthrin, Cypermethrin, Bifenthrin, Lufenuron, Triflumoron, Tefluthrin, Tebupirimphos, Ethiprole, Cyazypyr, Thiacloprid, Acetamiprid, Dinotefuran, Avermectin, Methiocarb, Spirodiclofen, Spirotetramat; Maize Fungicides: Fenitropan, Thiram, Prothioconazole, Tebuconazole, Trifloxystrobin; Rice Herbicides: Butachlor, Propanil, Azimsulfuron, Bensulfuron, Cyhalofop, Daimuron, Fentrazamide, Imazosulfuron, Mefenacet, Oxaziclomefone, Pyrazosulfuron, Pyributicarb, Quinclorac, Thiobencarb, Indanofan, Flufenacet, Fentrazamide, Halosulfuron, Oxaziclomefone, Benzobicyclon, Pyrifthalid, Penoxsulam, Bispyribac, Oxadiargyl, Ethoxysulfuron, Pretilachlor, Mesotrione, Tefuryltrione, Oxadiazone, Fenoxaprop, Pymisulfan; Rice Insecticides: Diazinon, Fenitrothion, Fenobucarb, Monocrotophos, Benfuracarb, Buprofezin, Dinotefuran, Fipronil, Imidacloprid, Isoprocarb, Thiacloprid, Chromafenozide, Thiacloprid, Dinotefuran, Clothianidin, Ethiprole, Flubendiamide, Rynaxypyr, Deltamethrin, Acetamiprid, Thiamethoxam, Cyazypyr, Spinosad, Spinotoram, Emamectin-Benzoate, Cypermethrin, Chlorpyrifos, Cartap, Methamidophos, Etofenprox, Triazophos, 4-[[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Carbofuran, Benfuracarb; Rice Fungicides: Thiophanate-methyl, Azoxystrobin, Carpropamid, Edifenphos, Ferimzone, Iprobenfos, Isoprothiolane, Pencycuron, Probenazole, Pyroquilon, Tricyclazole, Trifloxystrobin, Diclocymet, Fenoxanil, Simeconazole, Tiadinil; Cotton Herbicides: Diuron, Fluometuron, MSMA, Oxyfluorfen, Prometryn, Trifluralin, Carfentrazone, Clethodim, Fluazifop-butyl, Glyphosate, Norflurazon, Pendimethalin, Pyriothiobac-sodium, Trifloxysulfuron, Tepraloxym, Glufosinate, Flumioxazin, Thidiazuron; Cotton Insecticides: Acephate, Aldicarb, Chlorpyrifos, Cypermethrin, Deltamethrin, Malathion, Monocrotophos, Abamectin, Acetamiprid, Emamectin Benzoate, Imidacloprid, Indoxacarb, Lambda-Cyhalothrin, Spinosad, Thiodicarb, Gamma-Cyhalothrin, Spiromesifen, Pyridalyl, Flonicamid, Flubendiamide, Triflumuron, Rynaxypyr, Beta-Cyfluthrin, Spirotetramat, Clothianidin, Thiamethoxam, Thiacloprid, Dinotefuran, Flubendiamide, Cyazypyr, Spinosad, Spinotoram, gamma Cyhalothrin, 4-[[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Thiodicarb, Avermectin, Flonicamid, Pyridalyl, Spiromesifen, Sulfoxaflor, Profenophos, Thiazophos, Endosulfan; Cotton Fungicides: Etridiazole, Metalaxyl, Quintozene; Soybean Herbicides: Alachlor, Bentazone, Trifluralin, Chlorimuron-Ethyl, Cloransulam-Methyl, Fenoxaprop, Fomesafen, Fluazifop, Glyphosate, Imazamox, Imazaquin, Imazethapyr, (S-)Metolachlor, Metribuzin, Pendimethalin, Tepraloxym, Glufosinate; Soybean Insecticides: Lambda-cyhalothrin, Methomyl, Parathion, Thiocarb, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinotefuran, Flubendiamide, Rynaxypyr, Cyazypyr, Spinosad, Spinotoram, Emamectin-Benzoate, Fipronil, Ethiprole, Deltamethrin, beta-Cyfluthrin, gamma and lambda Cyhalothrin, 4-[[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Spirotetramat, Spirodiclofen, Triflumuron, Flonicamid, Thiodicarb, beta-Cyfluthrin; Soybean Fungicides: Azoxystrobin, Cyproconazole, Epoxiconazole, Flutriafol, Pyraclostrobin, Tebuconazole, Trifloxystrobin, Prothioconazole, Tetraconazole; Sugarbeet Herbicides: Chloridazon, Desmedipham, Ethofumesate, Phenmedipham, Triallate, Clopyralid, Fluazifop, Lenacil, Metamitron, Quinmerac, Cycloxydim, Triflusulfuron, Tepraloxym, Quizalofop; Sugarbeet Insecticides: Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinotefuran, Deltamethrin, beta-Cyfluthrin, gamma/lambda Cyhalothrin, 4-[[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Tefluthrin, Rynaxypyr, Cyazypyr, Fipronil, Carbofuran; Canola Herbicides: Clopyralid, Diclofop, Fluazifop, Glufosinate, Glyphosate, Metazachlor, Trifluralin Ethametsulfuron, Quinmerac, Quizalofop, Clethodim, Tepraloxym

dim; Canola Fungicides: Azoxystrobin, Carbendazim, Fludioxonil, Iprodione, Prochloraz, Vinclozolin; Canola Insecticides: Carbofuran organophosphates, Pyrethroids, Thiocloprid, Deltamethrin, Imidacloprid, Clothianidin, Thiamethoxam, Acetamiprid, Dinotofuran, β -Cyfluthrin, gamma and lambda Cyhalothrin, tau-Fluvaleriate, Ethiprole, Spinosad, Spinotram, Flubendiamide, Rynaxypyr, Cyazypyr, 4-[[[6-Chlorpyridin-3-yl)methyl]](2,2-difluorethyl)amino]furan-2(5H)-on.

[0266] In some embodiments the herbicide is Atrazine, Bromacil, Diuron, Chlorsulfuron, Metsulfuron, Thifensulfuron Methyl, Tribenuron, Acetochlor, Dicamba, Isoxaflutole, Nicosulfuron, Rimsulfuron, Pyriithiobac-sodium, Flumioxazin, Chlorimuron-Ethyl, Metribuzin, Quizalofop, S-metolachlor, Hexazinone or combinations thereof.

[0267] In some embodiments the insecticide is Esfenvalerate, Chlorantraniliprole, Methomyl, Indoxacarb, Oxamyl or combinations thereof.

Pesticidal and insecticidal activity

[0268] "Pest" includes but is not limited to, insects, fungi, bacteria, nematodes, mites, and ticks. Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc., particularly Lepidoptera and Coleoptera.

[0269] Those skilled in the art will recognize that not all compounds are equally effective against all pests. Compounds of the embodiments display activity against insect pests, which may include economically important agronomic, forest, greenhouse, nursery ornamentals, food and fiber, public and animal health, domestic and commercial structure, household and stored product pests.

[0270] Larvae of the order Lepidoptera include, but are not limited to, armyworms, cutworms, loopers and heliothines in the family Noctuidae *Spodoptera frugiperda* JE Smith (fall armyworm); *S. exigua* Hübner (beet armyworm); *S. litura* Fabricius (tobacco cutworm, cluster caterpillar); *Mamestra configurata* Walker (bertha armyworm); *M. brassicae* Linnaeus (cabbage moth); *Agrotis ipsilon* Hufnagel (black cutworm); *A. orthogonia* Morrison (western cutworm); *A. subterranea* Fabricius (granulate cutworm); *Alabama argillacea* Hübner (cotton leaf worm); *Trichoplusia ni* Hübner (cabbage looper); *Pseudoplusia includens* Walker (soybean looper); *Anticarsia gemmatilis* Hübner (velvetbean caterpillar); *Hypena scabra* Fabricius (green cloverworm); *Heliothis virescens* Fabricius (tobacco budworm); *Pseudaletia unipuncta* Haworth (armyworm); *Athetis mindara* Barnes and McDunnough (rough skinned cutworm); *Euxoa messoria* Harris (darksided cutworm); *Earias insulana* Boisduval (spiny bollworm); *E. vittella* Fabricius (spotted bollworm); *Helicoverpa armigera* Hübner (American bollworm); *H. zea* Boddie (corn earworm or cotton bollworm); *Melanchra picta* Harris (zebra caterpillar); *Egira (Xylomyges) curialis* Grote (citrus cutworm); borers, casebearers, webworms, coneworms, and skeletonizers from the family Pyralidae *Ostrinia nubilalis* Hübner (European corn borer); *Amyelois transitella* Walker (naval orangeworm); *Anagasta kuehniella* Zeller (Mediterranean flour moth); *Cadra cautella* Walker (almond moth); *Chilo suppressalis* Walker (rice stem borer); *C. partellus*, (sorghum borer); *Corcyra cephalonica* Stainton (rice moth); *Crambus caliginosellus* Clemens (corn root webworm); *C. teterrellus* Zincken (bluegrass webworm); *Cnaphalocrocis medinalis* Guenée (rice leaf roller); *Desmia funeralis* Hübner (grape leaf folder); *Diaphania hyalinata* Linnaeus (melon worm); *D. nitidalis* Stoll (pickworm); *Diatraea grandiosella* Dyar (southwestern corn borer), *D. saccharalis* Fabricius (surgarcane borer); *Eoreuma loftini* Dyar (Mexican rice borer); *Ephestia elutella* Hübner (tobacco (cacao) moth); *Galleria mellonella* Linnaeus (greater wax moth); *Herpetogramma licarsialis* Walker (sod webworm); *Homoeosoma electellum* Hulst (sunflower moth); *Elastopalpus lignosellus* Zeller (lesser cornstalk borer); *Achroia grisella* Fabricius (lesser wax moth); *Loxostege sticticalis* Linnaeus (beet webworm); *Orthaga thyrisalis* Walker (tea tree web moth); *Maruca testulalis* Geyer (bean pod borer); *Plodia interpunctella* Hübner (Indian meal moth); *Scirpophaga incertulas* Walker (yellow stem borer); *Udea rubigalis* Guenée (celery leaf tier); and leafrollers, budworms, seed worms and fruit worms in the family Tortricidae *Acleris gloverana* Walsingham (Western blackheaded budworm); *A. variana* Fernald (Eastern blackheaded budworm); *Archips argyrospila* Walker (fruit tree leaf roller); *A. rosana* Linnaeus (European leaf roller); and other *Archips* species, *Adoxophyes orana* Fischer von Rösslerstamm (summer fruit tortrix moth); *Cochylis hospes* Walsingham (banded sunflower moth); *Cydia latiferreana* Walsingham (filbertworm); *C. pomonella* Linnaeus (coding moth); *Platynota flavedana* Clemens (variegated leafroller); *P. stultana* Walsingham (omnivorous leafroller); *Lobesia botrana* Denis & Schiffermüller (European grape vine moth); *Spilonota ocellana* Denis & Schiffermüller (eyespotted bud moth); *Endopiza viteana* Clemens (grape berry moth); *Eupoecilia ambiguella* Hübner (vine moth); *Bonagota salubricola* Meyrick (Brazilian apple leafroller); *Grapholita molesta* Busck (oriental fruit moth); *Suleima helianthana* Riley (sunflower bud moth); *Argyrotaenia spp.*; *Choristoneura spp.*

[0271] Selected other agronomic pests in the order Lepidoptera include, but are not limited to, *Alsophila pometaria* Harris (fall cankerworm); *Anarsia lineatella* Zeller (peach twig borer); *Anisota senatoria* J.E. Smith (orange striped oakworm); *Antheraea pernyi* Guérin-Ménéville (Chinese Oak Tussah Moth); *Bombyx mori* Linnaeus (Silkworm); *Bucculatrix thurberiella* Busck (cotton leaf perforator); *Colias eurytheme* Boisduval (alfalfa caterpillar); *Datana integerrima* Grote & Robinson (walnut caterpillar); *Dendrolimus sibiricus* Tschetwerikov (Siberian silk moth), *Ennomos subsignaria* Hübner (elm spanworm); *Erannis tiliaria* Harris (linden looper); *Euproctis chrysorrhoea* Linnaeus (browntail moth); *Harrisina*

americana Guérin-Méneville (grapeleaf skeletonizer); *Hemileuca oliviae* Cockrell (range caterpillar); *Hyphantria cunea* Drury (fall webworm); *Keiferia lycopersicella* Walsingham (tomato pinworm); *Lambdina fiscellaria fiscellaria* Hulst (Eastern hemlock looper); *L. fiscellaria lugubrosa* Hulst (Western hemlock looper); *Leucoma salicis* Linnaeus (satin moth); *Lymantria dispar* Linnaeus (gypsy moth); *Manduca quinquemaculata* Haworth (five spotted hawk moth, tomato hornworm); *M. sexta* Haworth (tomato hornworm, tobacco hornworm); *Operophtera brumata* Linnaeus (winter moth); *Paleacrita vernata* Peck (spring cankerworm); *Papilio cresphontes* Cramer (giant swallowtail orange dog); *Phryganidia californica* Packard (California oakworm); *Phyllocnistis citrella* Stainton (citrus leafminer); *Phyllonorycter blancardella* Fabricius (spotted tentiform leafminer); *Pieris brassicae* Linnaeus (large white butterfly); *P. rapae* Linnaeus (small white butterfly); *P. napi* Linnaeus (green veined white butterfly); *Platyptilia carduidactyla* Riley (artichoke plume moth); *Plutella xylostella* Linnaeus (diamondback moth); *Pectinophora gossypiella* Saunders (pink bollworm); *Pontia protodice* Boisduval and Leconte (Southern cabbageworm); *Sabulodes aegrotata* Guenée (omnivorous looper); *Schizura concinna* J.E. Smith (red humped caterpillar); *Sitotroga cerealella* Olivier (Angoumois grain moth); *Thaumetopoea pityocampa* Schiffermüller (pine processionary caterpillar); *Tineola bisselliella* Hummel (webbing clothesmoth); *Tuta absoluta* Meyrick (tomato leafminer); *Yponomeuta padella* Linnaeus (ermine moth); *Heliothis subflexa* Guenée; *Malacosoma* spp. and *Orgyia* spp.

[0272] Of interest are larvae and adults of the order Coleoptera including weevils from the families Anthribidae, Bruchidae and Curculionidae (including, but not limited to: *Anthonomus grandis* Boheman (boll weevil); *Lissorhoptrus oryzophilus* Kuschel (rice water weevil); *Sitophilus granarius* Linnaeus (granary weevil); *S. oryzae* Linnaeus (rice weevil); *Hypera punctata* Fabricius (clover leaf weevil); *Cylindrocopturus adspersus* LeConte (sunflower stem weevil); *Smicronyx fulvus* LeConte (red sunflower seed weevil); *S. sordidus* LeConte (gray sunflower seed weevil); *Sphenophorus maidis* Chittenden (maize billbug)); flea beetles, cucumber beetles, rootworms, leaf beetles, potato beetles and leafminers in the family Chrysomelidae (including, but not limited to: *Leptinotarsa decemlineata* Say (Colorado potato beetle); *Diabrotica virgifera virgifera* LeConte (western corn rootworm); *D. barberi* Smith and Lawrence (northern corn rootworm); *D. undecimpunctata howardi* Barber (southern corn rootworm); *Chaetocnema pulicaria* Melsheimer (corn flea beetle); *Phyllotreta cruciferae* Goeze (Crucifer flea beetle); *Phyllotreta striolata* (stripped flea beetle); *Colaspis brunnea* Fabricius (grape colaspis); *Oulema melanopus* Linnaeus (cereal leaf beetle); *Zygogramma exclamationis* Fabricius (sunflower beetle)); beetles from the family Coccinellidae (including, but not limited to: *Epilachna varivestis* Mulsant (Mexican bean beetle)); chafers and other beetles from the family Scarabaeidae (including, but not limited to: *Popillia japonica* Newman (Japanese beetle); *Cyclocephala borealis* Arrow (northern masked chafer, white grub); *C. immaculata* Olivier (southern masked chafer, white grub); *Rhizotrogus majalis* Razoumowsky (European chafer); *Phyllophaga crinita* Burmeister (white grub); *Ligyris gibbosus* De Geer (carrot beetle)); carpet beetles from the family Dermestidae; wireworms from the family Elateridae, *Eleodes* spp., *Melanotus* spp.; *Conoderus* spp.; *Limonius* spp.; *Agriotes* spp.; *Ctenicera* spp.; *Aeolus* spp.; bark beetles from the family Scolytidae and beetles from the family Tenebrionidae.

[0273] Adults and immatures of the order Diptera are of interest, including leafminers *Agromyza parvicornis* Loew (corn blotch leafminer); midges (including, but not limited to: *Contarinia sorghicola* Coquillett (sorghum midge); *Mayetiola destructor* Say (Hessian fly); *Sitodiplosis mosellana* Géhin (wheat midge); *Neolasioptera murfeldtiana* Felt, (sunflower seed midge)); fruit flies (Tephritidae), *Oscinella frit* Linnaeus (fruit flies); maggots (including, but not limited to: *Delia platura* Meigen (seedcorn maggot); *D. coarctata* Fallen (wheat bulb fly) and other *Delia* spp., *Meromyza americana* Fitch (wheat stem maggot); *Musca domestica* Linnaeus (house flies); *Fannia canicularis* Linnaeus, *F. femoralis* Stein (lesser house flies); *Stomoxys calcitrans* Linnaeus (stable flies)); face flies, horn flies, blow flies, *Chrysomya* spp.; *Phormia* spp. and other muscoid fly pests, horse flies *Tabanus* spp.; bot flies *Gastrophilus* spp.; *Oestrus* spp.; cattle grubs *Hypoderma* spp.; deer flies *Chrysops* spp.; *Melophagus ovinus* Linnaeus (keds) and other *Brachycera*, mosquitoes *Aedes* spp.; *Anopheles* spp.; *Culex* spp.; black flies *Prosimulium* spp.; *Simulium* spp.; biting midges, sand flies, sciarids, and other *Nematocera*.

[0274] Included as insects of interest are adults and nymphs of the orders Hemiptera and Homoptera such as, but not limited to, adelgids from the family Adelgidae, plant bugs from the family Miridae, cicadas from the family Cicadidae, leafhoppers, *Empoasca* spp.; from the family Cicadellidae, planthoppers from the families Cixiidae, Flatidae, Fulgoroidea, Issidae and Delphacidae, treehoppers from the family Membracidae, psyllids from the family Psyllidae, whiteflies from the family Aleyrodidae, aphids from the family Aphididae, phylloxera from the family Phylloxeridae, mealybugs from the family Pseudococcidae, scales from the families Asterolecanidae, Coccidae, Dactylopiidae, Diaspididae, Eriococcidae, Ortheziidae, Phoenicococcidae and Margarodidae, lace bugs from the family Tingidae, stink bugs from the family Pentatomidae, cinch bugs, *Blissus* spp.; and other seed bugs from the family Lygaeidae, spittlebugs from the family Cercopidae squash bugs from the family Coreidae and red bugs and cotton stainers from the family Pyrrhocoridae.

[0275] Agronomically important members from the order Homoptera further include, but are not limited to: *Acyrtosiphon pisum* Harris (pea aphid); *Aphis craccivora* Koch (cowpea aphid); *A. fabae* Scopoli (black bean aphid); *A. gossypii* Glover (cotton aphid, melon aphid); *A. maidiradicis* Forbes (corn root aphid); *A. pomi* De Geer (apple aphid); *A. spiraeicola* Patch (spirea aphid); *Aulacorthum solani* Kalténbach (foxglove aphid); *Chaetosiphon fragaefolii* Cockerell (strawberry aphid); *Diuraphis noxia* Kurdjumov/Mordvilko (Russian wheat aphid); *Dysaphis plantaginea* Paaserini (rosy apple aphid); *Eriosoma lanigerum* Hausmann (woolly apple aphid); *Brevicoryne brassicae* Linnaeus (cabbage aphid); *Hyalopterus*

pruni Geoffroy (mealy plum aphid); *Lipaphis erysimi* Kaltenbach (turnip aphid); *Metopolophium dirrhodum* Walker (cereal aphid); *Macrosiphum euphorbiae* Thomas (potato aphid); *Myzus persicae* Sulzer (peach-potato aphid, green peach aphid); *Nasonovia ribisnigri* Mosley (lettuce aphid); *Pemphigus* spp. (root aphids and gall aphids); *Rhopalosiphum maidis* Fitch (corn leaf aphid); *R. padi* Linnaeus (bird cherry-oat aphid); *Schizaphis graminum* Rondani (greenbug); *Sipha flava* Forbes (yellow sugarcane aphid); *Sitobion avenae* Fabricius (English grain aphid); *Therioaphis maculata* Buckton (spotted alfalfa aphid); *Toxoptera aurantii* Boyer de Fonscolombe (black citrus aphid) and *T. citricida* Kirkaldy (brown citrus aphid); *Adelges* spp. (adelgids); *Phylloxera devastatrix* Pergande (pecan phylloxera); *Bemisia tabaci* Gennadius (tobacco whitefly, sweetpotato whitefly); *B. argentifolii* Bellows & Perring (silverleaf whitefly); *Dialeurodes citri* Ashmead (citrus whitefly); *Trialeurodes abutiloneus* (bandedwinged whitefly) and *T. vaporariorum* Westwood (greenhouse whitefly); *Empoasca fabae* Harris (potato leafhopper); *Laodelphax striatellus* Fallen (smaller brown planthopper); *Macrolestes quadrilineatus* Forbes (aster leafhopper); *Nephotettix cincticeps* Uhler (green leafhopper); *N. nigropictus* Stål (rice leafhopper); *Nilaparvata lugens* Stål (brown planthopper); *Peregrinus maidis* Ashmead (corn planthopper); *Sogatella furcifera* Horvath (white-backed planthopper); *Sogatodes orizicola* Muir (rice delphacid); *Typhlocyba pomaria* McAtee (white apple leafhopper); *Erythroneoura* spp. (grape leafhoppers); *Magicicada septendecim* Linnaeus (periodical cicada); *Icerya purchasi* Maskell (cottony cushion scale); *Quadraspidiotus perniciosus* Comstock (San Jose scale); *Planococcus citri* Risso (citrus mealybug); *Pseudococcus* spp. (other mealybug complex); *Cacopsylla pyricola* Foerster (pear psylla); *Trioza diospyri* Ashmead (persimmon psylla).

[0276] Agronomically important species of interest from the order Hemiptera include, but are not limited to: *Acrosternum hilare* Say (green stink bug); *Anasa tristis* De Geer (squash bug); *Blissus leucopterus leucopterus* Say (chinch bug); *Corythuca gossypii* Fabricius (cotton lace bug); *Cyrtopeltis modesta* Distant (tomato bug); *Dysdercus suturellus* Herrich-Schäffer (cotton stainer); *Euschistus servus* Say (brown stink bug); *E. variolarius* Palisot de Beauvois (one-spotted stink bug); *Graptostethus* spp. (complex of seed bugs); *Leptoglossus corculus* Say (leaf-footed pine seed bug); *Lygus lineolaris* Palisot de Beauvois (tarnished plant bug); *L. hesperus* Knight (Western tarnished plant bug); *L. pratensis* Linnaeus (common meadow bug); *L. rugulipennis* Poppius (European tarnished plant bug); *Lygocoris pabulinus* Linnaeus (common green capsid); *Nezara viridula* Linnaeus (southern green stink bug); *Oebalus pugnax* Fabricius (rice stink bug); *Oncopectus fasciatus* Dallas (large milkweed bug); *Pseudatomoscelis seriatus* Reuter (cotton fleahopper).

[0277] Furthermore, embodiments may be effective against Hemiptera such, *Calocoris norvegicus* Gmelin (strawberry bug); *Orthops campestris* Linnaeus; *Plesiocoris rugicollis* Fallen (apple capsid); *Cyrtopeltis modestus* Distant (tomato bug); *Cyrtopeltis notatus* Distant (suckfly); *Spanagonicus albofasciatus* Reuter (whitemarked fleahopper); *Diaphnocoris chlorionis* Say (honeylocust plant bug); *Labopidicola allii* Knight (onion plant bug); *Pseudatomoscelis seriatus* Reuter (cotton fleahopper); *Adelphocoris rapidus* Say (rapid plant bug); *Poecilocapsus lineatus* Fabricius (four-lined plant bug); *Nysius ericae* Schilling (false chinch bug); *Nysius raphanus* Howard (false chinch bug); *Nezara viridula* Linnaeus (Southern green stink bug); *Eurygaster* spp.; *Coreidae* spp.; *Pyrrhocoridae* spp.; *Tinidae* spp.; *Blostomatidae* spp.; *Reduviidae* spp. and *Cimicidae* spp.

[0278] Also included are adults and larvae of the order Acari (mites) such as *Aceria tosichella* Keifer (wheat curl mite); *Petrobia latens* Müller (brown wheat mite); spider mites and red mites in the family Tetranychidae, *Panonychus ulmi* Koch (European red mite); *Tetranychus urticae* Koch (two spotted spider mite); (*T. mcdanieli* McGregor (McDaniel mite); *T. cinnabarinus* Boisduval (carmine spider mite); *T. turkestanii* Ugarov & Nikolski (strawberry spider mite); flat mites in the family Tenuipalpidae, *Brevipalpus lewisi* McGregor (citrus flat mite); rust and bud mites in the family Eriophyidae and other foliar feeding mites and mites important in human and animal health, i.e., dust mites in the family Epidermoptidae, follicle mites in the family Demodicidae, grain mites in the family Glycyphagidae, ticks in the order Ixodidae. *Ixodes scapularis* Say (deer tick); *I. holocyclus* Neumann (Australian paralysis tick); *Dermacentor variabilis* Say (American dog tick); *Amblyomma americanum* Linnaeus (lone star tick) and scab and itch mites in the families Psoroptidae, Pyemotidae and Sarcoptidae.

[0279] Insect pests of the order Thysanura are of interest, such as *Lepisma saccharina* Linnaeus (silverfish); *Thermobia domestica* Packard (firebrat).

[0280] Additional arthropod pests covered include: spiders in the order Araneae such as *Loxosceles reclusa* Gertsch and Mulaik (brown recluse spider) and the *Latrodectus mactans* Fabricius (black widow spider) and centipedes in the order Scutigermorpha such as *Scutigera coleoptrata* Linnaeus (house centipede).

[0281] Insect pest of interest include the superfamily of stink bugs and other related insects including but not limited to species belonging to the family Pentatomidae (*Nezara viridula*, *Halyomorpha halys*, *Piezodorus guildini*, *Euschistus servus*, *Acrosternum hilare*, *Euschistus heros*, *Euschistus tristigmus*, *Acrosternum hilare*, *Dichelops furcatus*, *Dichelops melacanthus*, and *Bagrada hilaris* (*Bagrada* Bug)), the family Plataspidae (*Megacopta cribraria* - Bean plataspid) and the family Cydnidae (*Scaptocoris castanea* - Root stink bug) and Lepidoptera species including but not limited to: diamond-back moth, e.g., *Helicoverpa zea* Boddie; soybean looper, e.g., *Pseudoplusia includens* Walker and velvet bean caterpillar e.g., *Anticarsia gemmatalis* Hübner.

[0282] Methods for measuring pesticidal activity are well known in the art. See, for example, Czaplá and Lang, (1990) J. Econ. Entomol. 83:2480-2485; Andrews, et al., (1988) Biochem. J. 252:199-206; Marrone, et al., (1985) J. of Economic

Entomology 78:290-293 and US Patent Number 5,743,477. Generally, the protein is mixed and used in feeding assays. See, for example Marrone, et al., (1985) J. of Economic Entomology 78:290-293. Such assays can include contacting plants with one or more pests and determining the plant's ability to survive and/or cause the death of the pests.

[0283] Nematodes include parasitic nematodes such as root-knot, cyst and lesion nematodes, including *Heterodera* spp., *Meloidogyne* spp. and *Globodera* spp.; particularly members of the cyst nematodes, including, but not limited to, *Heterodera glycines* (soybean cyst nematode); *Heterodera schachtii* (beet cyst nematode); *Heterodera avenae* (cereal cyst nematode) and *Globodera rostochiensis* and *Globodera pallida* (potato cyst nematodes). Lesion nematodes include *Pratylenchus* spp.

Seed Treatment

[0284] To protect and to enhance yield production and trait technologies, seed treatment options can provide additional crop plan flexibility and cost effective control against insects, weeds and diseases. Seed material can be treated with one or more of the insecticidal proteins or polypeptides disclosed herein. For e.g., such seed treatments can be applied on seeds that contain a transgenic trait including transgenic corn, soy, brassica, cotton or rice. Combinations of one or more of the insecticidal proteins or polypeptides disclosed herein and other conventional seed treatments are contemplated. Seed material can be treated, typically surface treated, with a composition comprising combinations of chemical or biological herbicides, herbicide safeners, insecticides, fungicides, germination inhibitors and enhancers, nutrients, plant growth regulators and activators, bactericides, nematocides, avicides and/or molluscicides. These compounds are typically formulated together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. The coatings may be applied by impregnating propagation material with a liquid formulation or by coating with a combined wet or dry formulation. Examples of the various types of compounds that may be used as seed treatments are provided in The Pesticide Manual: A World Compendium, C.D.S. Tomlin Ed., Published by the British Crop Production Council.

[0285] Some seed treatments that may be used on crop seed include, but are not limited to, one or more of abscisic acid, acibenzolar-S-methyl, avermectin, amitrol, azaconazole, azospirillum, azadirachtin, azoxystrobin, *Bacillus* spp. (including one or more of cereus, firmus, megaterium, pumilis, sphaericus, subtilis and/or thuringiensis species), bradyrhizobium spp. (including one or more of betae, canariense, elkanii, iriomotense, japonicum, liaonigense, pachyrhizi and/or yuanmingense), captan, carboxin, chitosan, clothianidin, copper, cyazypyr, difenoconazole, etidiazole, fipronil, fludioxonil, fluoxastrobin, fluquinconazole, flurazole, fluxofenim, harpin protein, imazalil, imidacloprid, ipconazole, iso-flavenoids, lipo-chitooligosaccharide, mancozeb, manganese, maneb, mfenoxam, metalaxyl, metconazole, myclobutanil, PCNB, penflufen, penicillium, penthiopyrad, permethrin, picoxystrobin, prothioconazole, pyraclostrobin, rynaxypyr, S-metolachlor, saponin, sedaxane, TCMTB, tebuconazole, thiabendazole, thiamethoxam, thiocarb, thiram, tolclofos-methyl, triadimenol, trichoderma, trifloxystrobin, triticonazole and/or zinc. PCNB seed coat refers to EPA Registration Number 00293500419, containing quintozen and terrazole. TCMTB refers to 2-(thiocyanomethylthio) benzothiazole.

[0286] Seed varieties and seeds with specific transgenic traits may be tested to determine which seed treatment options and application rates may complement such varieties and transgenic traits in order to enhance yield. For example, a variety with good yield potential but head smut susceptibility may benefit from the use of a seed treatment that provides protection against head smut, a variety with good yield potential but cyst nematode susceptibility may benefit from the use of a seed treatment that provides protection against cyst nematode, and so on. Likewise, a variety encompassing a transgenic trait conferring insect resistance may benefit from the second mode of action conferred by the seed treatment, a variety encompassing a transgenic trait conferring herbicide resistance may benefit from a seed treatment with a safener that enhances the plants resistance to that herbicide, etc. Further, the good root establishment and early emergence that results from the proper use of a seed treatment may result in more efficient nitrogen use, a better ability to withstand drought and an overall increase in yield potential of a variety or varieties containing a certain trait when combined with a seed treatment.

Methods for killing an insect pest and controlling an insect population

[0287] In some embodiments methods are provided for killing an insect pest, comprising contacting the insect pest, either simultaneously or sequentially, with an insecticidally-effective amount of a recombinant PtIP-50 polypeptide and PtIP-65 polypeptide. In some embodiments methods are provided for killing an insect pest, comprising contacting the insect pest with an insecticidally-effective amount of a recombinant pesticidal protein and/or PtIP-65 polypeptide.

[0288] In some embodiments methods are provided for controlling an insect pest population, comprising contacting the insect pest population, either simultaneously or sequentially, with an insecticidally-effective amount of a recombinant PtIP-50 polypeptide and PtIP-65 polypeptide. In some embodiments methods are provided for controlling an insect pest population, comprising contacting the insect pest population with an insecticidally-effective amount of a recombinant PtIP-50 polypeptide and/or PtIP-65 polypeptide. As used herein, "controlling a pest population" or "controls a pest" refers

to any effect on a pest that results in limiting the damage that the pest causes. Controlling a pest includes, but is not limited to, killing the pest, inhibiting development of the pest, altering fertility or growth of the pest in such a manner that the pest provides less damage to the plant, decreasing the number of offspring produced, producing less fit pests, producing pests more susceptible to predator attack or deterring the pests from eating the plant.

[0289] In some embodiments methods are provided for controlling an insect pest population resistant to a pesticidal protein, comprising contacting the insect pest population, either simultaneously or sequentially, with an insecticidally-effective amount of a recombinant PtIP-50 polypeptide and a recombinant PtIP-65 polypeptide. In some embodiments methods are provided for controlling an insect pest population resistant to a pesticidal protein, comprising contacting the insect pest population with an insecticidally-effective amount of a recombinant PtIP-50 polypeptide and/or the PtIP-65 polypeptide.

[0290] In some aspects methods are disclosed for protecting a plant from an insect pest, comprising expressing in the plant or cell thereof at least one recombinant polynucleotide encoding a PtIP-50 polypeptide and a recombinant PtIP-65 polypeptide. In some aspects methods are disclosed for protecting a plant from an insect pest, comprising expressing in the plant or cell thereof a recombinant polynucleotide encoding PtIP-50 polypeptide and/or PtIP-65 polypeptide.

[0291] In some aspects methods are disclosed for protecting a plant from an insect pest, comprising expressing in the plant or cell thereof at least one recombinant polynucleotide encoding a PtIP-50 polypeptide and a recombinant PtIP-65 polypeptide. In some aspects methods are disclosed for protecting a plant from an insect pest, comprising expressing in the plant or cell thereof a recombinant polynucleotide encoding a PtIP-50 polypeptide.

Insect Resistance Management (IRM) Strategies

[0292] Expression of *B. thuringiensis* δ -endotoxins in transgenic corn plants has proven to be an effective means of controlling agriculturally important insect pests (Perlak, *et al.*, 1990; 1993). However, insects have evolved that are resistant to *B. thuringiensis* δ -endotoxins expressed in transgenic plants. Such resistance, should it become widespread, would clearly limit the commercial value of germplasm containing genes encoding such *B. thuringiensis* δ -endotoxins.

[0293] One way to increasing the effectiveness of the transgenic insecticides against target pests and contemporaneously reducing the development of insecticide-resistant pests is to use provide non-transgenic (i.e., non-insecticidal protein) refuges (a section of non-insecticidal crops/ corn) for use with transgenic crops producing a single insecticidal protein active against target pests. The United States Environmental Protection Agency (epa.gov/oppbpd/biopesticides/pips/bt_corn_refuge_2006.htm, which can be accessed using the www prefix) publishes the requirements for use with transgenic crops producing a single Bt protein active against target pests. In addition, the National Corn Growers Association, on their website: (ncga.com/insect-resistance-management-fact-sheet-bt-corn, which can be accessed using the www prefix) also provides similar guidance regarding refuge requirements. Due to losses to insects within the refuge area, larger refuges may reduce overall yield.

[0294] Another way of increasing the effectiveness of the transgenic insecticides against target pests and contemporaneously reducing the development of insecticide-resistant pests would be to have a repository of insecticidal genes that are effective against groups of insect pests and which manifest their effects through different modes of action.

[0295] Expression in a plant of two or more insecticidal compositions toxic to the same insect species, each insecticide being expressed at efficacious levels would be another way to achieve control of the development of resistance. This is based on the principle that evolution of resistance against two separate modes of action is far more unlikely than only one. Roush, for example, outlines two-toxin strategies, also called "pyramiding" or "stacking," for management of insecticidal transgenic crops. (The Royal Society. *Phil. Trans. R. Soc. Lond. B.* (1998) 353:1777-1786). Stacking or pyramiding of two different proteins each effective against the target pests and with little or no cross-resistance can allow for use of a smaller refuge. The US Environmental Protection Agency requires significantly less (generally 5%) structured refuge of non-Bt corn be planted than for single trait products (generally 20%). There are various ways of providing the IRM effects of a refuge, including various geometric planting patterns in the fields and in-bag seed mixtures, as discussed further by Roush.

[0296] In some embodiments the PtIP-50 polypeptide and PtIP-65 polypeptide of the disclosure are useful as an insect resistance management strategy in combination (i.e., pyramided) with other pesticidal proteins include but are not limited to Bt toxins, *Xenorhabdus* sp. or *Photorhabdus* sp. insecticidal proteins.

[0297] Provided are methods of controlling Lepidoptera and/or Coleoptera insect infestation(s) in a transgenic plant that promote insect resistance management, comprising expressing in the plant at least two different insecticidal proteins having different modes of action.

[0298] In some embodiments the methods of controlling Lepidoptera and/or Coleoptera insect infestation in a transgenic plant and promoting insect resistance management the at least one of the insecticidal proteins comprise a PtIP-50 polypeptide and PtIP-65 polypeptide insecticidal to insects in the order Lepidoptera and/or Coleoptera.

[0299] In some embodiments the methods of controlling Lepidoptera and/or Coleoptera insect infestation in a transgenic

plant and promoting insect resistance management comprise expressing at least one of the PtIP-50 polypeptide and/or a PtIP-65 polypeptide, insecticidal to insects in the order Lepidoptera and/or Coleoptera.

[0300] In some embodiments the methods of controlling Lepidoptera and/or Coleoptera insect infestation in a transgenic plant and promoting insect resistance management comprise expressing in the transgenic plant a PtIP-50 polypeptide and PtIP-65 polypeptide and a Cry protein insecticidal to insects in the order Lepidoptera and/or Coleoptera having different modes of action.

[0301] In some embodiments the methods of controlling Lepidoptera and/or Coleoptera insect infestation in a transgenic plant and promoting insect resistance management comprise expressing in the transgenic plant a PtIP-50 polypeptide and/or a PtIP-65 polypeptide and a Cry protein insecticidal to insects in the order Lepidoptera and/or Coleoptera having different modes of action.

[0302] Also disclosed are methods of reducing likelihood of emergence of Lepidoptera and/or Coleoptera insect resistance to transgenic plants expressing in the plants insecticidal proteins to control the insect species, comprising expression of a PtIP-50 polypeptide and a PtIP-65 polypeptide insecticidal to the insect species in combination with a second insecticidal protein to the insect species having different modes of action.

[0303] Also disclosed are means for effective Lepidoptera and/or Coleoptera insect resistance management of transgenic plants, comprising co-expressing at high levels in the plants two or more insecticidal proteins toxic to Lepidoptera and/or Coleoptera insects but each exhibiting a different mode of effectuating its killing activity, wherein the two or more insecticidal proteins comprise a PtIP-50 polypeptide and PtIP-65 polypeptide and a Cry protein. Also disclosed are means for effective Lepidoptera and/or Coleoptera insect resistance management of transgenic plants, comprising co-expressing at high levels in the plants two or more insecticidal proteins toxic to Lepidoptera and/or Coleoptera insects but each exhibiting a different mode of effectuating its killing activity, wherein the two or more insecticidal proteins comprise a PtIP-50 polypeptide and/or PtIP-65 polypeptide and a Cry protein.

[0304] In addition, methods are disclosed for obtaining regulatory approval for planting or commercialization of plants expressing proteins insecticidal to insects in the order Lepidoptera and/or Coleoptera, comprising the step of referring to, submitting or relying on insect assay binding data showing that the PtIP-50 polypeptide and the PtIP-65 polypeptide does not compete with binding sites for Cry proteins in such insects. In addition, methods are disclosed for obtaining regulatory approval for planting or commercialization of plants expressing proteins insecticidal to insects in the order Lepidoptera and/or Coleoptera, comprising the step of referring to, submitting or relying on insect assay binding data showing that the PtIP-50 polypeptide and/or the PtIP-65 polypeptide does not compete with binding sites for Cry proteins in such insects.

Methods for Increasing Plant Yield

[0305] Methods for increasing plant yield are disclosed. The methods comprise providing a plant or plant cell expressing a polynucleotide encoding the pesticidal polypeptide sequence disclosed herein and growing the plant or a seed thereof in a field infested with a pest against which the polypeptide has pesticidal activity. In some aspects, the polypeptide has pesticidal activity against a Lepidopteran, Coleopteran, Dipteran, Hemipteran or nematode pest, and the field is infested with a Lepidopteran, Hemipteran, Coleopteran, Dipteran or nematode pest.

[0306] As defined herein, the "yield" of the plant refers to the quality and/or quantity of biomass produced by the plant. "Biomass" as used herein refers to any measured plant product. An increase in biomass production is any improvement in the yield of the measured plant product. Increasing plant yield has several commercial applications. For example, increasing plant leaf biomass may increase the yield of leafy vegetables for human or animal consumption. Additionally, increasing leaf biomass can be used to increase production of plant-derived pharmaceutical or industrial products. An increase in yield can comprise any statistically significant increase including, but not limited to, at least a 1% increase, at least a 3% increase, at least a 5% increase, at least a 10% increase, at least a 20% increase, at least a 30%, at least a 50%, at least a 70%, at least a 100% or a greater increase in yield compared to a plant not expressing the pesticidal sequence.

[0307] In specific methods, plant yield is increased as a result of improved pest resistance of a plant expressing a PtIP-50 polypeptide and a PtIP-65 polypeptide disclosed herein. Expression of the PtIP-50 polypeptide and the PtIP-65 polypeptide results in a reduced ability of a pest to infest or feed on the plant, thus improving plant yield.

Methods of Processing

[0308] Further disclosed are methods of processing a plant, plant part or seed to obtain a food or feed product from a plant, plant part or seed comprising a PtIP-50 polypeptide and a PtIP-65 polypeptide. The plants, plant parts or seeds provided herein, can be processed to yield oil, protein products and/or by-products that are derivatives obtained by processing that have commercial value. Non-limiting examples include transgenic seeds comprising a nucleic acid molecule encoding a PtIP-50 polypeptide and a PtIP-65 polypeptide which can be processed to yield soy oil, soy products

and/or soy by-products.

[0309] "Processing" refers to any physical and chemical methods used to obtain any soy product and includes, but is not limited to, heat conditioning, flaking and grinding, extrusion, solvent extraction or aqueous soaking and extraction of whole or partial seeds

[0310] The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTALS

Example 1 - Identification of an insecticidal protein active from the Fern, *Asplenium australasicum*, (PS-8566)

[0311] The insecticidal proteins PtIP-50Aa and PtIP-65Aa were identified by protein purification, mass spectroscopy (MS) and PCR cloning from *Asplenium australasicum*, PS-8566 as follows.

[0312] *Asplenium australasicum* was collected by a collaborator and assigned identification number PS-8566. A voucher specimen of *Asplenium australasicum* was sent to the Delaware State University Herbarium where the identity was verified. PS-8566 was collected, flash frozen in liquid N₂ and stored at -80° C. After storage it was ground to a fine powder at liquid N₂ temperatures with a Geno Ball Mill (SPEX, Metuchen, NJ). To extract protein, 20 ml of 50 mM Tris buffer, pH 8.0, 150 mM KCl, 2.5 mM EDTA, 1.5% polyvinylpyrrolidone (PVPP) and protease inhibitor cocktail (Roche Diagnostics, Germany) was added to every 5 g fresh weight of tissue. The homogenate was centrifuged to remove cell debris, filtered through 0.22 um filters and desalted using 10 ml Zeba Spin Desalting columns (Thermo Scientific, IL.)

[0313] Bioassays against the three pest species, Soybean Looper (SBL) (*Pseudoplusia includens*), Corn Earworm (CEW) (*Helicoverpa zea*) and European Corn Borer (ECB) (*Ostrinia nubilalis*) were conducted using the desalted protein extract overlaid onto an agar-based Lepidoptera diet (Southland Products Inc., Lake Village, AR) in a 96-well plate format. Six replicates were used per sample. Samples were allowed to dry on top of the diet and two to five neonate insects were placed into each well of the treated plate. After four days of incubation at 27°C larvae were scored for mortality or severity of stunting. The scores were recorded numerically as dead (3), severely stunted (2) (little or no growth but alive and equivalent to a 1st instar larvae), stunted (1) (growth to second instar but not equivalent to controls), or normal (0). Subjecting the sample to proteinase K and heat treatments resulted in loss of activity indicating that the sample was proteinaceous in nature. Bioassay results are shown in Table 7.

Table 7

Activity of <i>A. australasicum</i> protein extract against lepidoptera larvae			
		Ave. Score	Ave. Score after Proteinase K/Heat
Neonate	Soybean Looper	3	0
	Corn Earworm	2	0
	European Corn Borer	1.5	0

[0314] For protein purification, PS-8566 was ground to a fine powder at liquid N₂ temperatures with a Geno Ball Mill (SPEX, Metuchen, NJ). Protein was extracted in 50 mM Tris buffer, pH 8.0, 150 mM KCl, 2.5 mM EDTA, 1.5% PVPP and protease inhibitor cocktail (Roche Diagnostics, Germany). The extracted material was centrifuged to remove cell debris, filtered through 0.22 um filters and desalted using a HiPrep 26/10 desalting column (GE, Piscataway, NJ). The desalted fractions were assayed against CEW and SBL as described above. The active protein fraction pool was loaded onto a 5 ml HiTrap Q column (GE, Piscataway, N.J.) and eluted with a linear (30 CV) gradient from 0 M to 0.6 M NaCl in 50 mM Tris, pH 8.0. The active fractions were combined, concentrated by Amicon molecular weight cutoff filtration (Millipore, Billerica, MA), and loaded onto a HiLoad™ Superdex™ 200, 16/60 Size exclusion column (GE, Piscataway, N.J.) and eluted with 50 mM Tris buffer, pH 8.0, 150 mM NaCl. Fractions active against SBL and CEW were combined, concentrated and then further diluted with 50 mM Tris buffer, pH 8 to reduce the salt concentration and loaded onto a Mono Q® 5/50 column (GE, Piscataway N.J.). Fractions eluted from the Mono Q® 5/50 column with a 0 to 0.7M NaCl gradient over 200 CV were assayed against SBL and CEW. Based on LDS-PAGE the active fraction contained two protein bands at approximately 89 kDa and 35 kDa. The two bands were designated with internal names to be used hereafter. The protein representing the 89 kDa band was named PtIP-50Aa and the protein representing the 35 kDa band was named PtIP-65Aa.

[0315] Protein sequencing and identification was performed by MS analysis after protein digestion with trypsin. Proteins for MS identification were obtained after running the sample on an LDS-PAGE gel stained with Brilliant Blue G-250 Stain. The two bands of interest were excised from the gel, de-stained, reduced with dithiothreitol and then alkylated with iodoacetamide. Following overnight digestion with trypsin, the samples were submitted for LCMS analysis. Liquid chro-

matography-tandem mass spectrometry (LC-MSMS) analysis for tryptically-digested peptides was conducted using electrospray on a QToF Premiere™ mass spectrometer (Waters®, Milford, MA) coupled with a NanoAcquity™ nano-LC system (Waters®, Milford, MA) with a gradient from 2% acetonitrile, 0.1% formic acid to 60% acetonitrile, 0.1% formic acid.

5 [0316] The resulting LCMS data were analyzed using Protein Lynx Global Server (Waters®, Milford, MA) to generate DeNovo sequence data. The amino acid sequences were BLAST (Basic Local Alignment Search Tool; Altschul, et al., (1993) J. Mol. Biol. 215:403-410; see also ncbi.nlm.nih.gov/BLAST/, which can be accessed using the www prefix) searched against public and DUPONT-PIONEER internal databases that included plant protein sequences. Amino acid sequences were aligned with proteins in a proprietary DUPONT-PIONEER plant protein database.

10 Example 2 - Transcriptomic Sequencing of PtIP-50 and PtIP-65

[0317] A transcriptome for *Asplenium australasicum*, PS-8566 was prepared as follows. Total RNAs were isolated from frozen tissues by use of the Qiagen® RNeasy® kit for total RNA isolation. Sequencing libraries from the resulting total RNAs were prepared using the TruSeq™ mRNA-Seq kit and protocol from Illumina®, Inc. (San Diego, CA). Briefly, mRNAs were isolated via attachment to oligo(dT) beads, fragmented to a mean size of 180 nt, reverse transcribed into cDNA by random hexamer prime, end repaired, 3' A-tailed, and ligated with Illumina® indexed TruSeq™ adapters. Ligated cDNA fragments were PCR amplified using Illumina® TruSeq™ primers and purified PCR products were checked for quality and quantity on the Agilent Bioanalyzer® DNA 7500 chip. Post quality and quantity assessment, 100 ng of the transcript library was normalized by treatment with Duplex Specific Nuclease (DSN) (Evrogen®, Moscow, Russia). Normalization was accomplished by addition of 200 mM Hepes buffer, followed by heat denaturation and five hour anneal at 68°C. Annealed library was treated with 2 ul of DSN enzyme for 25 minutes, purified by Qiagen® MinElute® columns according to manufacturer protocols, and amplified twelve cycles using Illumina® adapter specific primers. Final products were purified with Ampure® XP beads (Beckman Genomics, Danvers, MA) and checked for quality and quantity on the

25 Agilent Bioanalyzer® DNA 7500 chip.
[0318] Normalized transcript libraries were sequenced according to manufacturer protocols on the Illumina® Genome Analyzer Ix. Each library was hybridized to two flowcell lanes and amplified, blocked, linearized and primer hybridized using the Illumina clonal cluster generation process on cBot®. Sequencing was completed on the Genome Analyzer Ix, generating sixty million 75 bp paired end reads per normalized library.

30 [0319] Peptide sequences identified for PtIP-50Aa by LC-MS/MS/MS sequencing (described in Example 1) were searched against the protein sequences predicted by open reading frames (ORFs) from the internal transcriptome for PS-8566 assemblies. The peptides gave a perfect match to a transcript corresponding to PtIP-50Aa. Amino acid sequence stretches identified for PtIP-65Aa by LC-MS/MS/MS sequencing were searched against the proteins predicted by open reading frames (ORFs) of the contig assemblies. The peptides gave perfect matches to another transcript corresponding to PtIP-65Aa.

35 [0320] The coding sequences were used to design the following primers to clone the PtIP-50Aa coding sequence: CGAATTCGAGCACATATGGCCGCC (95KD N-T NdeI for) and GATTCCGGATCCTTATACGGCGACTCTGG (95KD C-T BamHI REV). This clone was produced by polymerase chain reaction using the HF Advantage® PCR kit (Clontech™, 1290 Terra Bella Ave. Mountain View, CA 94043) and the total RNA from *Asplenium australasicum* as a template. The cloned sequence was confirmed by sequencing.

40 [0321] The coding sequences were used to design the following primers to clone the PtIP-65Aa coding sequence: GGATACACCTCATATGGCGTTGGTGATCGG (36KD N-T NdeI for) and GTGCGGGATCCCTACTGAGTGGTGATGTCAATCGCG (36KD C-T BamHI REV). This clone was produced by polymerase chain reaction using the HF Advantage® PCR kit (Clontech™, 1290 Terra Bella Ave. Mountain View, CA 94043) and the total RNA from *Asplenium australasicum* as a template. The cloned sequence was confirmed by sequencing.

45 Example 3: - Identification of PtIP-50 and PtIP-65 Homologs

[0322] Gene identities may be determined by conducting BLAST (Basic Local Alignment Search Tool; Altschul, et al., (1993) J. Mol. Biol. 215:403-410; see also ncbi.nlm.nih.gov/BLAST/, which can be accessed using the www prefix) searches under default parameters for similarity to sequences. The polynucleotide sequences for PtIP-50Aa and PtIP-65Aa were analyzed. Gene identities conducted by BLAST in a DUPONT PIONEER internal transcriptomes database of ferns and other primitive plants identified multiple homologs for both PtIP-50Aa and PtIP-65Aa within a range of homologies. Table 8 shows the Plant Sample ID #, fern or other primitive plant species, internal clone designation and the respective sequence identifiers for the PtIP-65 polypeptide homologs and polynucleotides encoding the PtIP-50 homologs. Table 9 shows the Plant Sample ID #, fern or other primitive plant species, internal clone designation and the respective sequence identifiers for the PtIP-65 polypeptide homologs and polynucleotides encoding the PtIP-50 homologs.

EP 3 102 684 B1

Table 8

Plant Sample ID	Species	name
PS-8566	<i>Asplenium australasicum</i>	PtIP-65Aa
PS-9140	<i>Asplenium x kenzoii</i> Sa. Kurata	PtIP-65Hc
PS-8568	<i>Polypodium musifolium</i>	PtIP-65Ba
		PtIP-65Ha
PS-9319	<i>Polypodium punctatum</i> 'Serratum'	PtIP-65Bb
		PtIP-65Gc
		PtIP-65Hd
PS-7140	<i>Adiantum pedatum</i> L.	PtIP-65Ca
PS-9135	<i>Platycterium bifurcatum</i>	PtIP-65Ga
		PtIP-65Hb
PS-8837	<i>Nephrolepis falcata</i>	PtIP-65He
		PtIP-65Hf
PS-9145	<i>Ophioglossum pendulum</i>	PtIP-65Gb
PS-9427	<i>Colysis wrightii</i> 'Monstifera'	PtIP-65Gd
PS-7897	<i>Colysis wrightii</i>	PtIP-65Ge
		PtIP-65Hk
PS-8780	<i>Selaginella kraussiana</i> 'Variegata'	PtIP-65Hh
		PtIP-65Hj
PS-7896	<i>Selaginella victoriae</i>	PtIP-65Hg
PS-8582	<i>Lycopodium phlegmaria</i>	PtIP-65Fa
PS-9141	<i>Huperzia salvinoides</i>	PtIP-65Fb

Table 9

Plant Sample ID	Species	name
PS-8566	<i>Asplenium australasicum</i>	PtIP-50Aa
		PtIP-50Fb
PS-8564	<i>Asplenium nidus</i>	PtIP-50Fo
PS-9140	<i>Asplenium x kenzoii</i> Sa. Kurata	PtIP-50Fs
		PtIP-50Fr
		PtIP-50Gc
PS-8568	<i>Polypodium musifolium</i>	PtIP-50Ba
PS-9319	<i>Polypodium punctatum</i> 'Serratum'	PtIP-50Bc
		PtIP-50Fe
		PtIP-50Gb
		PtIP-50Fh
		PtIP-50Fg
		PtIP-50Ff

EP 3 102 684 B1

(continued)

5
10
15
20
25

Plant Sample ID	Species	name
PS-7140	Adiantum pedatum L.	PtIP-50Bb
		PtIP-50Ft
		PtIP-50Fa
PS-9135	Platycerium bifurcatum	PtIP-50Fd
		PtIP-50Ga
PS-8837	Nephrolepis falcata	PtIP-50Fk
		PtIP-50Fi
		PtIP-50Fj
		PtIP-50Gd
PS-9427	Colysis wrightii 'Monstifera'	PtIP-50Fn
PS-7897	Colysis wrightii	PtIP-50Fi
		PtIP-50Fq
PS-9321	Bolbitis cladorrhizans	PtIP-50Bd
		PtIP-50Fp
PS-9537	Blechnum brasiliense 'Crispum'	PtIP-50Fm

Table 10

30
35
40
45

	PtIP-50Ba	PtIP-50Bb	PtIP-50Bc	PtIP-50Bd	PtIP-50Fa	PtIP-50Fb	PtIP-50Fd	PtIP-50Fe	PtIP-50Ff	PtIP-50Fg	PtIP-50Fh	PtIP-50Fi	PtIP-50Fj	PtIP-50Fk
PtIP-50Aa	86.3	84.8	83.0	88.4	46.3	48.4	46.9	48.9	51.3	46.1	47.7	48.4	50.9	46.1
PtIP-50Ba	-	79.3	87.5	81.3	45.7	47.9	45.7	49.4	51.2	45.4	46.4	46.9	49.2	46.3
PtIP-50Bb	-	-	76.5	85.9	47.4	48.9	47.4	49.6	52.9	46.9	48.4	49.7	52.2	47.6
PtIP-50Bc	-	-	-	78.1	45.0	47.1	45.9	47.9	48.8	45.2	45.8	45.3	48.6	45.2
PtIP-50Bd	-	-	-	-	47.3	49.3	48.2	49.7	52.6	47.4	49.2	48.6	51.8	46.3
PtIP-50Fa	-	-	-	-	-	44.0	39.5	42.6	58.4	40.9	40.2	49.9	55.6	40.9
PtIP-50Fb	-	-	-	-	-	-	52.3	71.7	46.9	51.2	51.4	44.5	45.4	77.9
PtIP-50Fd	-	-	-	-	-	-	-	52.5	44.8	67.1	64.2	42.8	43.8	50.7
PtIP-50Fe	-	-	-	-	-	-	-	-	46.6	52.4	52.1	44.6	44.6	69.7

50
55

EP 3 102 684 B1

	PtIP-50Ff	-	-	-	-	-	-	-	-	-	45.0	44.0	56.6	64.1	43.8
	PtIP-50Fg	-	-	-	-	-	-	-	-	-	-	65.9	42.8	43.5	50.0
5	PtIP-50Fh	-	-	-	-	-	-	-	-	-	-	-	43.5	44.0	50.0
	PtIP-50Fi	-	-	-	-	-	-	-	-	-	-	-	-	60.8	42.1
	PtIP-50Fj	-	-	-	-	-	-	-	-	-	-	-	-	-	42.4
	PtIP-50Fk	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	PtIP-50Fl	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Fm	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Fn	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Fo	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	PtIP-50Fp	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Fq	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Fr	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Fs	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	PtIP-50Ft	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Ga	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Gb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	PtIP-50Gc	-	-	-	-	-	-	-	-	-	-	-	-	-	-

25 Table 10 cont.

	PtIP-50Fl	PtIP-50Fm	PtIP-50Fn	PtIP-50Fo	PtIP-50Fp	PtIP-50Fq	PtIP-50Fr	PtIP-50Fs	PtIP-50Ft	PtIP-50Ga	PtIP-50Gb	PtIP-50Gc	PtIP-50Gd	
30	PtIP-50Aa	47.6	46.6	48.1	45.1	41.4	49.4	46.2	47.7	51.1	32.9	33.4	35.2	33.5
	PtIP-50Ba	47.8	46.4	47.8	44.8	40.7	49.1	45.7	47.8	50.5	31.5	32.1	33.4	32.3
35	PtIP-50Bb	48.0	47.8	49.1	45.4	41.7	50.7	47.1	47.8	52.3	32.4	33.3	33.8	33.7
	PtIP-50Bc	46.0	45.9	47.1	44.0	40.5	47.4	44.8	46.1	49.6	32.2	32.8	33.3	32.4
	PtIP-50Bd	48.4	47.7	49.1	45.6	41.2	51.3	47.4	48.1	52.9	33.3	34.2	34.8	33.6
40	PtIP-50Fa	42.2	40.3	43.7	40.7	37.5	57.4	38.9	43.8	45.4	33.2	32.6	33.8	31.5
	PtIP-50Fb	68.4	52.6	71.9	76.0	40.6	46.3	51.0	88.8	59.4	33.1	31.7	32.4	33.2
	PtIP-50Fd	51.9	83.5	54.0	49.2	38.1	43.4	84.3	52.5	56.6	32.4	31.8	31.6	31.0
	PtIP-50Fe	81.8	52.8	85.2	66.5	42.2	46.1	51.7	71.4	60.6	33.0	32.4	32.5	32.8
45	PtIP-50Ff	45.4	45.3	47.5	44.2	39.4	89.8	44.1	46.2	49.7	32.1	32.7	32.8	31.6
	PtIP-50Fg	50.8	66.0	53.3	48.6	38.4	44.3	65.7	51.4	56.7	31.0	29.6	32.3	30.4
	PtIP-50Fh	51.7	65.3	53.3	48.6	39.1	43.4	63.9	51.0	55.3	31.0	30.5	30.8	31.0
	PtIP-50Fi	42.6	42.9	44.3	41.0	40.2	55.5	42.7	43.8	46.7	32.6	32.7	33.3	30.8
	PtIP-50Fj	44.4	43.2	45.5	41.9	38.7	62.5	42.6	44.1	49.2	31.1	31.7	31.3	30.7
50	PtIP-50Fk	67.5	51.7	70.7	76.4	38.3	43.4	50.3	76.5	55.4	33.5	32.2	32.8	32.8
	PtIP-50Fl	-	52.0	86.2	63.3	40.9	45.2	51.1	68.0	57.8	32.3	31.3	32.4	32.5
	PtIP-50Fm	-	-	54.3	51.2	39.4	44.6	84.7	53.3	56.5	32.6	31.8	31.8	31.8

EP 3 102 684 B1

PtIP-50Fn	-	-	-	66.2	41.2	45.9	52.7	71.2	60.9	33.3	32.5	32.5	33.4
PtIP-50Fo	-	-	-	-	37.9	44.2	48.7	79.4	54.1	32.5	32.6	32.7	32.7
PtIP-50Fp	-	-	-	-	-	39.8	38.7	40.4	41.9	39.3	39.6	39.9	38.3
PtIP-50Fq	-	-	-	-	-	-	44.2	45.7	48.2	31.3	32.4	32.2	30.6
PtIP-50Fr	-	-	-	-	-	-	-	51.8	55.9	31.7	31.1	31.4	30.6
PtIP-50Fs	-	-	-	-	-	-	-	-	58.6	33.4	31.9	32.9	33.0
PtIP-50Ft	-	-	-	-	-	-	-	-	-	33.5	32.6	34.0	32.9
PtIP-50Ga	-	-	-	-	-	-	-	-	-	-	85.9	87.4	77.2
PtIP-50Gb	-	-	-	-	-	-	-	-	-	-	-	90.4	75.2
PtIP-50Gc	-	-	-	-	-	-	-	-	-	-	-	-	77.6

[0323] Moreover, tables summarizing the global identity are presented in Table 10 and Table 11. Percent identity values were calculated using ClustalW algorithm in the ALIGNX® module of the Vector NTI® Program Suite (Invitrogen™ Corporation, Carlsbad, Calif.) with all default parameters.

Table 11

	PtIP-65Ba	PtIP-65Bb	PtIP-65Ca	PtIP-65Fa	PtIP-65Fb	PtIP-65Ga	PtIP-65Gb	PtIP-65Gc	PtIP-65Gd	PtIP-65Ge	PtIP-65Ha	PtIP-65Hb
PtIP-65Aa	82.9	83.2	76.8	46.4	46.9	35.2	35.8	33.6	34.5	34.5	33.6	26.6
PtIP-65Ba	-	85.0	74.0	44.8	44.8	33.9	34.1	33.2	32.9	32.9	33.2	26.8
PtIP-65Bb	-	-	73.1	44.2	45.4	33.0	33.2	32.3	32.1	32.1	32.3	28.4
PtIP-65Ca	-	-	-	46.1	46.6	33.8	34.8	33.2	33.6	33.3	32.7	28.0
PtIP-65Fa	-	-	-	-	96.8	41.7	42.3	42.8	44.0	43.8	43.4	28.1
PtIP-65Fb	-	-	-	-	-	42.3	42.8	42.8	44.0	43.8	43.4	28.7
PtIP-65Ga	-	-	-	-	-	-	98.6	85.1	83.3	83.1	83.9	27.2
PtIP-65Gb	-	-	-	-	-	-	-	85.6	83.9	83.6	84.5	27.4
PtIP-65Gc	-	-	-	-	-	-	-	-	92.1	91.8	98.3	27.1
PtIP-65Gd	-	-	-	-	-	-	-	-	-	99.7	92.7	25.9
PtIP-65Ge	-	-	-	-	-	-	-	-	-	-	92.4	25.7
PtIP-65Ha	-	-	-	-	-	-	-	-	-	-	-	26.4
PtIP-65Hb	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65Hc	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65Hd	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65He	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65Hf	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65Hg	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65Hh	-	-	-	-	-	-	-	-	-	-	-	-

EP 3 102 684 B1

PtIP-65Hj	-	-	-	-	-	-	-	-	-	-	-	-
PtIP-65Hk	-	-	-	-	-	-	-	-	-	-	-	-

5

Table 11 cont.

10

15

20

25

30

35

40

	PtIP-65Hc	PtIP-65Hd	PtIP-65He	PtIP-65Hf	PtIP-65Hg	PtIP-65Hh	PtIP-65Hj	PtIP-65Hk
PtIP-65Aa	28.5	28.1	26.8	27.5	27.5	29.4	30.7	30.2
PtIP-65Ba	29.2	28.5	27.8	28.0	28.6	28.5	29.3	30.1
PtIP-65Bb	31.8	29.4	27.7	29.7	28.6	27.1	29.0	30.4
PtIP-65Ca	30.1	28.6	27.5	29.3	30.0	30.6	30.9	30.2
PtIP-65Fa	30.3	28.4	31.6	32.2	32.9	36.6	38.2	34.1
PtIP-65Fb	31.0	28.4	31.7	31.0	32.6	36.8	38.5	33.8
PtIP-65Ga	23.2	24.4	23.4	24.6	29.4	31.6	56.0	32.2
PtIP-65Gb	23.8	24.9	23.6	24.9	29.4	31.3	56.3	31.7
PtIP-65Gc	23.8	23.6	24.1	25.1	29.7	31.2	53.5	30.8
PtIP-65Gd	23.3	23.4	24.7	25.9	29.0	32.5	56.2	31.7
PtIP-65Ge	23.1	23.2	24.4	25.7	29.0	32.5	56.0	31.4
PtIP-65Ha	23.2	23.1	24.4	25.4	29.2	31.4	54.0	30.5
PtIP-65Hb	68.5	68.7	51.4	52.2	22.0	24.0	24.2	24.5
PtIP-65Hc	-	84.4	53.2	53.8	21.5	22.9	21.3	22.5
PtIP-65Hd	-	-	51.4	52.6	22.3	22.9	22.0	24.2
PtIP-65He	-	-	-	81.7	23.4	23.7	23.6	24.0
PtIP-65Hf	-	-	-	-	24.0	22.1	23.8	26.1
PtIP-65Hg	-	-	-	-	-	40.1	27.0	29.9
PtIP-65Hh	-	-	-	-	-	-	29.1	28.3
PtIP-65Hj	-	-	-	-	-	-	-	29.3
PtIP-65Hk	-	-	-	-	-	-	-	-

Example 4: Transient expression and insect bioassay on transient leaf tissues

45

50

55

[0324] To confirm activity of PtIP-50Aa and PtIP-65Aa as working together as a complex, both were cloned into a transient expression system under control of a viral promoter dMMV and AtUBQ10 respectively (Dav, et. al., (1999) Plant Mol. Biol. 40:771-782; Norris SR et al (1993) Plant Mol Biol. 21(5):895-906). PtIP-50Aa and PtIP-65Aa were infiltrated alone and co-infiltrated at a 1:1 ratio. The polynucleotide encoding PtIP-65Aa was cloned as a C-terminal HA tagged fusion vector for detection purposes resulting in the polynucleotide encoding the polypeptide PtIP-65Aa-2xHA tag. The agro-infiltration method of introducing an *Agrobacterium* cell suspension to plant cells of intact tissues so that reproducible infection and subsequent plant derived transgene expression may be measured or studied is well known in the art (Kapila, et. al., (1997) Plant Science 122:101-108). Briefly, the unifoliate stage of bush bean (common bean, *Phaseolus vulgaris*) or soybean (*Glycine max*), were agro-infiltrated with normalized bacterial cell cultures of test and control strains. Leaf discs were excised from each plantlet and infested with 2 neonates of Soy Bean Looper (SBL) (*Pseudoplusia includens*), Corn Earworm, (CEW) (*Helicoverpa zea*) or 4 neonates of European Corn Borer (ECB) (*Ostrinia nubilalis*) alone. Leaf discs from a control was generated with *Agrobacterium* containing only a DsRed2 fluorescence marker (Clontech™, 1290 Terra Bella Ave. Mountain View, CA 94043) expression vector. Leaf discs from a non-infiltrated plant was run as a second control. The consumption of green leaf tissue were scored after two (CEW) or three (ECB, SBL) days after infestation and given scores of 0 to 9. Neither the PtIP-50Aa polypeptide or the PtIP-65Aa-

EP 3 102 684 B1

2XHA tagged polypeptide alone showed insecticidal activity at the concentrations tested, however insecticidal activity was observed when the expression vectors for both proteins were co-infiltrated (Table 12). The transiently co-expressed PtIP-50Aa polypeptide and PtIP-65Aa-2xHA tag polypeptide protected leaf discs from consumption by the infested insects while total green tissue consumption was observed for the negative control and untreated tissue. Transient protein expression of both PtIP-50Aa and PtIP-65Aa-2xHA tagged was confirmed by mass spectrometry-based protein identification method using trypsinized protein extracts of infiltrated leaf tissues (Patterson, (1998) 10(22): 1-24, Current Protocol in Molecular Biology published by John Wiley & Son Inc) and by Western Analysis of the HA tag. These proteins were also active when run as a molecular stack in the bush bean transient assay system.

[0325] Experiments were run to test various tagged versions of both PtIP-50Aa (PtIP50Aa-GAAEPEA tag; MBP-rTEV-PtIP50Aa) and PtIP-65Aa (PtIP065Aa-GAAEPEA tag; PtIP65Aa-rTEV-2XHA tag; 2XHA tag-PtIP65Aa; 10 HIS-MBP-rTEV-PtIP65Aa) which showed that tagged versions of both proteins are still active and similar to untagged protein.

Table 12

Construct	Insect	Avg.	StdDEV
PtIP-50Aa + PtIP-65Aa-2XHA tag (1:1)	SBL	9	0
pVER	SBL	2.8	2.3
blank	SBL	1	0
PtIP-50Aa + PtIP-65Aa-2XHA tag (1:1)	CEW	9	0
pVER	CEW	1	0
blank	CEW	1	0
PtIP-50Aa + PtIP-65Aa-2XHA tag (1:1)	ECB	5.8	1
pVER	ECB	1.4	1
blank	ECB	1.4	1
Value			
Description			
1	leaf disk is greater than 90% consumed		
2	leaf disk is 70 - 80% consumed		
3	leaf disk is 60-70% consumed		
4	leaf disk is 50-60% consumed		
5	leaf disk is 40-50% consumed		
6	leaf disk is less than 30% consumed		
7	leaf disk is less than 10% consumed		
8	leaf disk has only a few pinholes		
9	leaf disk is untouched by the insect		

Example 5: Lepidoptera assays with purified proteins transiently expressed in Bush Bean Assay

[0326] Insecticidal activity bioassay screens were conducted with C-terminally HA tagged PtIP-65Aa-rTEV-2XHA, and PtIP-50Aa that were co-infiltrated into the bush bean transient system described above. The protein was purified from homogenized plant tissue using affinity beads targeting the 2xHA tag (Sigma-Aldrich A2095 Monoclonal Anti-HA-Agarose). The protein was tested in diet assay to evaluate the insecticidal protein effects on larvae of a diversity of *Lepidoptera*.

[0327] *Lepidoptera* feeding assays were conducted on an artificial diet containing PtIP-50Aa protein and PtIP-65Aa-rTEV-2XHA from 1:1 infiltrated Bush Bean. The PtIP-50Aa and PtIP-65Aa-rTEV-2XHA insecticidal proteins were tested for activity against Soy Bean Looper (SBL) (*Pseudoplusia includens*), Corn Earworm, (CEW) (*Helicoverpa zea*) and European Corn Borer (ECB) (*Ostrinia nubilalis*). For SBL, the proteins were incorporated into a Lepidopteran-specific

EP 3 102 684 B1

artificial diet (Southland Products) wells at a rate of 0, 0.06 ppm, 0.2 ppm, 0.63 ppm, 2 ppm, 6.3 ppm and 20 ppm. For CEW, the proteins were incorporated into a Lepidopteran-specific artificial diet (Southland Products) wells at a rate of 0, 0.16 ppm, 0.5 ppm, 1.58 ppm, 5.0 ppm, 15.8 ppm and 50 ppm. For ECB, the proteins were incorporated into a Lepidopteran-specific artificial diet (Southland Products) wells at a rate of 0, 0.38 ppm, 1.2 ppm, 3.8 ppm, 12.0 ppm, 38 ppm and 120 ppm. Neonate (2-3) larvae were placed in each well to feed for four days. Each bioassay was run with 4 duplicates at each dose. Results were expressed as positive for larvae reactions such as stunting and or mortality. Results were expressed as negative if the larvae were similar to the negative control that was fed diet to which the above buffer only has been applied. Larvae that had a size similar to controls were assigned a score of 0, those that showed slight stunting were scored 1, those that showed severe stunting were scored 2 and those that were dead were scored 3. The insecticidal activity of PtIP-50Aa and PtIP-65Aa-rTEV-2XHA combined in the Soy Bean Looper (SBL) (*Pseudoplusia includens*), Corn Earworm, (CEW) (*Helicoverpa zea*) and European Corn Borer (ECB) (*Ostrinia nubilalis*) is shown in Table 13. Table 13 shows the LC50 based on the concentration (ppm) of PtIP-50Aa.

Table 13

Insect		PtIP-50Aa ppm	Lower 95%CL	Upper 95%CL
CEW	LC50	16.73	12.50	23.18
ECB	LC50	>120		
SBL	LC50	0.9196	0.3099	2.578

Example 6: Co-Infiltration of PtIP-50 polypeptides and PtIP-65 polypeptides cloned from different Genera of ferns exhibit activity.

[0328] Polynucleotides encoding PtIP-50 polypeptide homologs and PtIP-65 polypeptide homologs were identified from several species of ferns as shown in Example 3 and some were cloned into the transient expression vector as described in Example 2 above. Polynucleotides encoding the homologs were cloned from one of three ferns, *Asplenium australasicum*, *Polypodium musifolium* and *Adiantum pedatum* (Table 14.) Each homolog was co-infiltrated 1:1 in the bush bean transient system in a mix and match experiment to determine activity and spectrum from each combination. Results from various combinations in the mix and match experiment show that PtIP-50 polypeptides and PtIP-65 polypeptides from different sources of ferns can have activity with each other (Table 15.) The spectrum of activity is broadened with the improvement in FAW activity with PtIP-65Ha.

Table 14

Fern	PtIP-50 Homolog	PtIP-65 Homolog
Asplenium australasicum	PtIP-50Aa	PtIP-65Aa
Polypodium musifolium	PtIP-50Ba	PtIP-65Ba
		PtIP-65Ha
Adiantum pedatum	PtIP-50Bb	PtIP-65Ca

Table 15

		bushbean transient activity			
PtIP-50 polypeptide	PtIP-65 polypeptide	CEW	ECB	FAW	SBL
PtIP-50Aa	PtIP-65Aa	8.3	6.3	1.6	8.9
PtIP-50Aa	PtIP-65Ba	8.5	1.3	1.8	6.9
PtIP-50Aa	PtIP-65Ca	8.0	8.4	1.6	9.0
PtIP-50Aa	PtIP-65Ha	5.8	1.2	1.0	8.5
PtIP-50Ba	PtIP-65Ba	8.8	1.6	1.0	6.3

(continued)

		bushbean transient activity			
PtIP-50 polypeptide	PtIP-65 polypeptide	CEW	ECB	FAW	SBL
PtIP-50Bb	PtIP-65Aa	8.4	6.4	1.0	9.0
PtIP-50Bb	PtIP-65Ba	8.3	2.2	1.0	5.3
PtIP-50Bb	PtIP-65Ca	8.4	9.0	1.0	9.0
PtIP-50Ga	PtIP-65Hb	6.8	7.8	1.8	8.0

Example 7: Baculovirus Expression of PtIP-50Aa and PtIP-65Aa polypeptides

[0329] The gene encoding PtIP-50Aa was synthesized and subcloned into a pFastBac™ Dual vector (Invitrogen®) with the PtIP-50Aa native stop codon (TAA). The gene encoding PtIP-65Aa-10XHis was subcloned with the stop codon removed for C-terminal translation of a 10x-histidine tag addition and the gene sequence of the histidine-tagged PtIP-65Aa. This vector was transformed into *DH10Bac* cells to generate baculovirus. These baculovirus were used to infect sf9 insect cells and incubated for 72 hours at 27°C. The infected insect cells were harvested by centrifugation. The cell culture pellet was suspended with 200 ml of lysis buffer (1XPBS, 10% glycerol, with protease inhibitor and benzonase) and incubated at 4°C for 5 min with stirring, then homogenizing twice. Chaps was added to lysate to a 1.0% final concentration and this was stirred at 4°C for 1 hour. The lysate was centrifuged at 16000 rpm for 20 min. The supernatant was saved and loaded onto six 2 ml Ni-NTA columns pre-equilibrated with Elute buffer (1XPBS, 10% glycerol). The columns were then eluted with 10 ml of Elute buffer consisting of 10, 20, 50, and 250 mM of imidazole. Samples were analyzed by SDS-PAGE. The fraction from the 250 mM imidazole elution were concentrated to 5 mL and loaded onto two PD-10 desalting columns (GE Life Sciences, Pittsburg, USA) pre-equilibrated with Elute buffer. 3 mL of Elute buffer was loaded on to each column and 2.5 mL of eluate collected from each column. The eluates from two desalting columns were combined. Lysates were assayed against SBL and CEW and were active when both PtIP-65Aa-10XHis and PtIP-50Aa were present (Table 16).

Table 16

Protein & expression	Insect	LC/IC	LC/IC50 ppm (4d)	Lower 95%CL	Upper 95%CL
PtIP-50Aa / 65Aa-10XHis	CEW	LC50	>100		
		IC50	22.24	12.56	47.10
	SBL	LC50	1.157	0.7220	1.811
		IC50	0.2992	0.1974	0.4297

Example 8: *Agrobacterium*-Mediated Transformation of Maize and Regeneration of Transgenic Plants

[0330] For *Agrobacterium*-mediated transformation of maize with PtIP-65 and PtIP-50 nucleotide sequences such as PtIP-50Aa and PtIP-65Aa, the method of Zhao can be used (US Patent Number 5,981,840 and PCT Patent Publication Number WO 1998/32326. Briefly, immature embryos are isolated from maize and the embryos contacted with a suspension of *Agrobacterium* under conditions whereby the bacteria are capable of transferring the nucleotide sequence (PtIP-50Aa, PtIP-65Aa) to at least one cell of at least one of the immature embryos (step 1: the infection step). In this step the immature embryos can be immersed in an *Agrobacterium* suspension for the initiation of inoculation. The embryos are co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step). The immature embryos can be cultured on solid medium following the infection step. Following this co-cultivation period an optional "resting" step is contemplated. In this resting step, the embryos are incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformation (step 3: resting step). The immature embryos can be cultured on solid medium with antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for the infected cells. Next, inoculated embryos are cultured on medium containing a selective agent and growing transformed callus is recovered (step 4: the selection step). The immature embryos are cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus is then regenerated into plants (step 5: the regeneration step), and calli grown on selective medium can be cultured on solid medium to regenerate the plants.

EP 3 102 684 B1

Example 9: Transformation and Regeneration of Soybean (Glycine max)

[0331] Transgenic soybean lines are generated by the method of particle gun bombardment (Klein et al., Nature (London) 327:70-73 (1987); U.S. Patent No. 4,945,050) using a BIORAD Biolistic PDS1000/He instrument and either plasmid or fragment DNA. The following stock solutions and media are used for transformation and regeneration of soybean plants:

Stock solutions:

[0332]

Sulfate 100 X Stock:

37.0 g $MgSO_4 \cdot 7H_2O$, 1.69 g $MnSO_4 \cdot H_2O$, 0.86 g $ZnSO_4 \cdot 7H_2O$, 0.0025 g $CuSO_4 \cdot 5H_2O$

Halides 100 X Stock:

30.0 g $CaCl_2 \cdot 2H_2O$, 0.083 g KI, 0.0025 g $CoCl_2 \cdot 6H_2O$

P, B, Mo 100X Stock:

18.5 g KH_2PO_4 , 0.62 g H_3BO_3 , 0.025 g $Na_2MoO_4 \cdot 2H_2O$

Fe EDTA 100X Stock:

3.724 g Na_2EDTA , 2.784 g $FeSO_4 \cdot 7H_2O$

2,4-D Stock:

10 mg/mL Vitamin

B5 vitamins, 1000X Stock:

100.0 g myo-inositol, 1.0 g nicotinic acid, 1.0 g pyridoxine HCl, 10 g thiamine.HCL.

Media (per Liter):

[0333]

SB199 Solid Medium:

1 package MS salts (Gibco/ BRL - Cat. No. 11117-066), 1 mL B5 vitamins 1000X stock, 30g Sucrose, 4 ml 2, 4-D (40 mg/L final concentration), pH 7.0, 2 gm Gelrite

SB1 Solid Medium:

1 package MS salts (Gibco/ BRL - Cat. No. 11117-066), 1 mL B5 vitamins 1000X stock, 31.5 g Glucose, 2 mL 2, 4-D (20 mg/L final concentration), pH 5.7, 8 g TC agar

SB196:

10 mL of each of the above stock solutions 1-4, 1 mL B5 Vitamin stock, 0.463 g $(NH_4)_2 SO_4$, 2.83 g KNO_3 , 1 mL 2,4 D stock, 1 g asparagine, 10 g Sucrose, pH 5.7

SB71-4:

Gamborg's B5 salts, 20 g sucrose, 5 g TC agar, pH 5.7.

SB103:

1 pk. Murashige & Skoog salts mixture, 1 mL B5 Vitamin stock, 750 mg $MgCl_2$ hexahydrate, 60 g maltose, 2 g gelrite, pH 5.7.

SB166:

SB103 supplemented with 5 g per liter activated charcoal.

Soybean Embryogenic Suspension Culture Initiation:

[0334] Pods with immature seeds from available soybean plants 45-55 days after planting are picked, removed from their shells and placed into a sterilized magenta box. The soybean seeds are sterilized by shaking them for 15 min in a 5% Clorox® solution with 1 drop of Ivory™ soap (i.e., 95 mL of autoclaved distilled water plus 5 mL Clorox® and 1 drop of soap, mixed well). Seeds are rinsed using 2 L sterile distilled water and those less than 3 mm are placed on individual microscope slides. The small end of the seed is cut and the cotyledons pressed out of the seed coat. Cotyledons are transferred to plates containing SB199 medium (25-30 cotyledons per plate) for 2 weeks, then transferred to SB1 for

EP 3 102 684 B1

2-4 weeks. Plates are wrapped with fiber tape. After this time, secondary embryos are cut and placed into SB196 liquid medium for 7 days.

Culture Conditions:

[0335] Soybean embryogenic suspension cultures (cv. 93Y21) were maintained in 50 mL liquid medium SB196 on a rotary shaker, 100 - 150 rpm, 26 °C on 16:8 h day/night photoperiod at light intensity of 80-100 $\mu\text{E}/\text{m}^2/\text{s}$. Cultures are subcultured every 7-14 days by inoculating up to ½ dime size quantity of tissue (clumps bulked together) into 50 mL of fresh liquid SB196.

Preparation of DNA for Bombardment:

[0336] In particle gun bombardment procedures it is possible to use purified 1) entire plasmid DNA; or 2) DNA fragments containing only the recombinant DNA expression cassette(s) of interest. For every seventeen bombardment transformations, 85 μL of suspension is prepared containing 1 to 90 picograms (pg) of plasmid DNA per base pair of each DNA plasmid. DNA plasmids or fragments are co-precipitated onto gold particles as follows. The DNAs in suspension are added to 50 μL of a 10 - 60 mg/mL 0.6 μm gold particle suspension and then combined with 50 μL CaCl_2 (2.5 M) and 20 μL spermidine (0.1 M). The mixture is vortexed for 5 sec, spun in a microfuge for 5 sec, and the supernatant removed. The DNA-coated particles are then washed once with 150 μL of 100% ethanol, vortexed and spun in a microfuge again, then resuspended in 85 μL of anhydrous ethanol. Five μL of the DNA-coated gold particles are then loaded on each macrocarrier disk.

Tissue Preparation and Bombardment with DNA:

[0337] Approximately 100 mg of two-week-old suspension culture is placed in an empty 60 mm X 15 mm petri plate and the residual liquid removed from the tissue using a pipette. The tissue is placed about 3.5 inches away from the retaining screen and each plate of tissue is bombarded once. Membrane rupture pressure is set at 650 psi and the chamber is evacuated to -28 inches of Hg. Following bombardment, the tissue from each plate is divided between two flasks, placed back into liquid media, and cultured as described above.

Selection of Transformed Embryos and Plant Regeneration:

[0338] After bombardment, tissue from each bombarded plate is divided and placed into two flasks of SB196 liquid culture maintenance medium per plate of bombarded tissue. Seven days post bombardment, the liquid medium in each flask is replaced with fresh SB196 culture maintenance medium supplemented with 100 ng/ml selective agent (selection medium). For selection of transformed soybean cells the selective agent used can be a sulfonyleurea (SU) compound with the chemical name, 2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazine-2-yl)aminocarbonyl) benzenesulfonamide (common names: DPX-W4189 and chlorsulfuron). Chlorsulfuron is the active ingredient in the DuPont sulfonyleurea herbicide, GLEAN®. The selection medium containing SU is replaced every two weeks for 8 weeks. After the 8 week selection period, islands of green, transformed tissue are observed growing from untransformed, necrotic embryogenic clusters. These putative transgenic events are isolated and kept in SB196 liquid medium with SU at 100 ng/ml for another 5 weeks with media changes every 1-2 weeks to generate new, clonally propagated, transformed embryogenic suspension cultures. Embryos spend a total of around 13 weeks in contact with SU. Suspension cultures are subcultured and maintained as clusters of immature embryos and also regenerated into whole plants by maturation and germination of individual somatic embryos.

[0339] Somatic embryos became suitable for germination after four weeks on maturation medium (1 week on SB166 followed by 3 weeks on SB103). They are then removed from the maturation medium and dried in empty petri dishes for up to seven days. The dried embryos are then planted in SB71-4 medium where they are allowed to germinate under the same light and temperature conditions as described above. Germinated embryos are transferred to potting medium and grown to maturity for seed production.

[0340] Leaf discs were excised from the transformed plants and tested for insecticidal activity of PtIP-50 and PtIP-65 against the Soy Bean Looper (SBL) (*Pseudoplusia includens*), Corn Earworm, (CEW) (*Helicoverpa zea*), European Corn Borer (ECB) (*Ostrinia nubilalis*) and Velvet Bean Caterpillar (VBC) (*Anticarsia gemmatilis*). The expected activity was observed for SBL, CEW and ECB, however activity was recorded in assay against VBC as well (Table 17).

Table 17

Event	DNA	VBC 3rd avg. score	SBL 3rd avg. score	SBL NEO avg. score	CEW NEO avg. score	FAW NEO avg. score	VBC 1st avg. score	ECB NEO avg. score
SOY 1985.3.1	PHP61857A	5.5	8.0	8.3	8.0	1.0	8.0	8.7
SOY 1987.2.1	PHP61857A	4.5	8.0	8.8	8.3	1.0		

Example 10: Mode of Action

[0341] PtIP-50 polypeptides and PtIP-65 polypeptides were evaluated for stability in the presence of midgut fluid extracts from *Pseudoplusia includens* to determine if their full length states represent pro-forms of the proteins and whether midgut proteolysis is required for activation to a toxic state in vivo.

[0342] The ability of the mixture of a PtIP-50 polypeptide and a PtIP-65 polypeptide to alter short circuit current (SCC) in isolated midguts from the *Lepidoptera*, *Anacarsia gemmatalis* was evaluated. Effects on the SCC in this system is considered consistent with pore formation, ion channel interaction, electrogenic transporter interaction, or possibly disruption of tight junctions that maintain diffusional barriers between midgut cells. An example of the effects of the mixture of the PtIP-50Aa and PtIP-65Aa polypeptides on SCC is shown in Figure 18. The ISCC reflects the transport activity and midgut structural integrity to maintain normal midgut function. The decline in ISCC following the addition of PtIP-50Aa and PtIP-65Aa polypeptides reflects the loss of ionic balance in the midgut.

[0343] The direct binding of the PtIP-50Aa and PtIP-65Aa polypeptides to *Pseudoplusia includens* brush border membrane vesicles was tested for target site identification. Specific binding, demonstrates the presence of a receptor for this binary. Heterologous competition with other insect-active toxins was tested to evaluate the competitive binding to the target site. Specific binding of PtIP-50Aa and PtIP-65Aa is illustrated in Figures 19 and 20a and 20b. Specific binding, demonstrates the presence of a receptor for this binary. Heterologous competition with other insect-active toxins was tested to evaluate the competitive binding to the target site. Specific binding of PtIP-50Aa and PtIP-65Aa is illustrated in Figures 19, 20a and 20b. Figure 19 shows a gel image of the specific binding of PtIP-50Aa and PtIP-65Aa to BBMV from *C.includens*. As the concentration of unlabeled PtIP-50Aa and PtIP-65Aa is increased, the likelihood that Alexa-labeled PtIP-50Aa and PtIP-65Aa can bind to the target receptor is decreased. The concentration at which the fluorescence signal from bound Alexa-PtIP-50Aa and PtIP-65Aa is reduced to 50% when compared to the signal measured in the absence of unlabeled PtIP-50Aa and PtIP-65Aa reflects the point at which half (50%) of the total receptors are occupied by unlabeled PtIP-50Aa and PtIP-65Aa and is referred to as the EC50 concentration. Densitometry results and EC50 determinations from the average three replicates are shown in Figures 20a and 20b. Figures 20a and 20b show the evaluation of EC50 values from densitometry of gel images for PtIP-50Aa and PtIP-65Aa binding to *C.includens* BBMVs. Figure 20a shows the average densitometry values for bound Alexa-PtIP-50Aa in the presence of different concentrations of unlabeled PtIP-50Aa and PtIP-65Aa normalized to the amount bound in the absence of unlabeled PtIP-50Aa and PtIP-65Aa. The solid line reflects the best fit of a square logistic equation to the data. The data are best fit by a two-component equation having EC50 values of 10 nM and 370 nM for the high affinity and low affinity components, respectively. Figure 20b shows the average densitometry values for bound Alexa- PtIP-65Aa in the presence of different concentrations of unlabeled PtIP-50Aa and PtIP-65Aa normalized to the amount bound in the absence of unlabeled PtIP-50Aa and PtIP-65Aa. The EC50 value from the fit is 14 nM.

Example 11: PtIP-50 & PtIP-65 Fusion Proteins

[0344] Fusion protein(s) of PtIP-50Aa and PtIP-65Aa Constructs on Excel Sheet) were created with a small synthetic linker between the two proteins and cloned into the Bush Bean transient system.

[0345] Two fusion proteins were made with PtIP-50Aa either N-terminal or C-terminal to PtIP-65Aa. After infiltration of bush bean leaf discs were infested with either CEW, ECB or SBL and assayed for 3 (CEW) or 4 (SBL, ECB) days. The results of the assay show that the fusion protein did protect the leaf discs from consumption and are a viable control option (Table 18).

EP 3 102 684 B1

Table 18

Fusion	
PtIP-65Aa/PtIP-50Aa	SBL (9.0) CEW (7.2)
PtIP-50Aa/PtIP-65Aa	SBL (8.8) CEW (8.7) ECB (6.1)

Example 12 - Particle bombardment Transformation and Regeneration of Transgenic Plants

[0346] Immature maize embryos from greenhouse donor plants are bombarded with a plasmid containing a nucleotide sequence encoding the insecticidal protein. The ears are husked and surface sterilized in 30% Clorox® bleach plus 0.5% Micro detergent for 20 minutes and rinsed two times with sterile water. The immature embryos are excised and placed embryo axis side down (scutellum side up), 25 embryos per plate, on 560Y medium for 4 hours and then aligned within the 2.5 cm target zone in preparation for bombardment. A plasmid vector DNA comprising the nucleotide sequence encoding the insecticidal protein operably linked to a promoter is precipitated onto 1.1 μm (average diameter) tungsten pellets using a CaCl₂ precipitation procedure as follows: 100 μl prepared tungsten particles in water; 10 μl (1 μg) DNA in Tris EDTA buffer (1 μg total DNA); 100 μl 2.5 M CaCl₂ and 10 μl 0.1 M spermidine.

[0347] Each reagent is added sequentially to the tungsten particle suspension, while maintained on the multitube vortexer. The final mixture is sonicated briefly and allowed to incubate under constant vortexing for 10 minutes. After the precipitation period, the tubes are centrifuged briefly, liquid removed, washed with 500 ml 100% ethanol and centrifuged for 30 seconds. Again the liquid is removed, and 105 μl 100% ethanol is added to the final tungsten particle pellet. For particle gun bombardment, the tungsten/DNA particles are briefly sonicated and 10 μl spotted onto the center of each macrocarrier and allowed to dry about 2 minutes before bombardment. The sample plates are bombarded at level #4 in a particle gun. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA

[0348] Following bombardment, the embryos are kept on 560Y medium for 2 days, then transferred to 560R selection medium containing 3 mg/liter Bialaphos, and subcultured every 2 weeks. After approximately 10 weeks of selection, selection-resistant callus clones are transferred to 288J medium to initiate plant regeneration. Following somatic embryo maturation (2-4 weeks), well-developed somatic embryos are transferred to medium for germination and transferred to the lighted culture room. Approximately 7-10 days later, developing plantlets are transferred to 272V hormone-free medium in tubes for 7-10 days until plantlets are well established. Plants are then transferred to inserts in flats (equivalent to 2.5" pot) containing potting soil and grown for 1 week in a growth chamber, subsequently grown an additional 1-2 weeks in the greenhouse, then transferred to classic 600 pots (1.6 gallon) and grown to maturity. Plants are monitored and scored for expression of a PtIP-50 polypeptide or a PtIP-65 polypeptide by assays known in the art, such as, for example, immunoassays and Western blotting.

[0349] Transgenic maize plants positive for expression of the insecticidal proteins are tested for pesticidal activity using standard bioassays known in the art. Such methods include, for example, root excision bioassays and whole plant bioassays. See, e.g., US Patent Application Publication Number US 2003/0120054 and International Publication Number WO 2003/018810.

[0350] Bombardment medium (560Y) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000.times.SIGMA-1511), 0.5 mg/l thiamine HCl, 120.0 g/l sucrose, 1.0 mg/l 2,4-D and 2.88 g/l L-proline (brought to volume with D-I H₂O following adjustment to pH 5.8 with KOH); 2.0 g/l Gelrite (added after bringing to volume with D-I H₂O) and 8.5 mg/l silver nitrate (added after sterilizing the medium and cooling to room temperature). Selection medium (560R) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000.times.SIGMA-1511), 0.5 mg/l thiamine HCl, 30.0 g/l sucrose and 2.0 mg/l 2,4-D (brought to volume with D-I H₂O following adjustment to pH 5.8 with KOH); 3.0 g/l Gelrite (added after bringing to volume with D-I H₂O) and 0.85 mg/l silver nitrate and 3.0 mg/l bialaphos (both added after sterilizing the medium and cooling to room temperature).

[0351] Plant regeneration medium (288J) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g nicotinic acid, 0.02 g/l thiamine HCL, 0.10 g/l pyridoxine HCL, and 0.40 g/l glycine brought to volume with polished D-I H₂O) (Murashige and Skoog, (1962) *Physiol. Plant.* 15:473), 100 mg/l myo-inositol, 0.5 mg/l zeatin, 60 g/l sucrose and 1.0 ml/l of 0.1 mM abscisic acid (brought to volume with polished D-I H₂O after adjusting to pH 5.6); 3.0 g/l Gelrite (added after bringing to volume with D-I H₂O) and 1.0 mg/l indoleacetic acid and 3.0 mg/l bialaphos (added after sterilizing the medium and cooling to 60°C). Hormone-free medium (272V) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g/l nicotinic acid, 0.02 g/l thiamine HCL, 0.10 g/l pyridoxine HCL

and 0.40 g/l glycine brought to volume with polished D-I H₂O), 0.1 g/l myo-inositol and 40.0 g/l sucrose (brought to volume with polished D-I H₂O after adjusting pH to 5.6) and 6 g/l bacto-agar (added after bringing to volume with polished D-I H₂O), sterilized and cooled to 60°C.

[0352] Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight; temperature is in degrees centigrade; and pressure is at or near atmospheric.

Claims

1. A recombinant PtIP-50 polypeptide, wherein the amino acid sequence of the PtIP-50 polypeptide has:

- a) at least 80% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Aa as shown in Figures 10a-10o;
- b) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc, or PtIP-50Bd as shown in Figures 10a-10o;
- c) at least 70% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Fb, PtIP-50Fs, PtIP-50Fo, PtIP-50Fk, PtIP-50Fe, PtIP-50FI, PtIP-50Fn, or PtIP-50Ft as shown in Figures 10a-10o;
- d) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Fd, PtIP-50Fm, PtIP-50Fr, PtIP-50Fg, or PtIP-50Fh as shown in Figures 10a-10o;
- e) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Ga, PtIP-50Gb, PtIP-50Gc, or PtIP-50Gd as shown in Figures 10a-10o; or
- f) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-50Fa, PtIP-50Ff, PtIP-50Fq, PtIP-50Fi, or PtIP-50Fj as shown in Figures 10a-10o;

wherein the PtIP-50 polypeptide has insecticidal activity in combination with a PtIP-65 polypeptide as defined in claim 3.

2. The recombinant PtIP-50 polypeptide of claim 1, wherein the PtIP-50 polypeptide:

(A) further comprises one or more characteristics selected from the group consisting of:

- a) a molecular weight of between about 80kDa and about 100kDa;
- b) an isoelectric point between about 4.4 and 5.2; and
- c) does not have chitinase activity;

(B) in combination with a PtIP-65 polypeptide:

- a) specifically binds to brush border membrane vesicles isolated from a Lepidoptera; or
- b) disrupts the ionic balance across brush border membrane vesicles isolated from a Lepidoptera; or

(C) is derived from a species of a fern of the Division Pteridophyta or a primitive plant of the Division Lycophyta; preferably wherein:

a) the fern is in the Genus:

- (i) *Asplenium*;
- (ii) *Blechnum*;
- (iii) *Bolbitis*;
- (iv) *Nephrolepis*;
- (v) *Polypodium*;
- (vi) *Platyserium*;
- (vii) *Colysis*;
- (viii) *Adiantaceae*; or
- (ix) *Ophioglossum*; or

b) the primitive plant is in the Genus:

- (i) *Selaginella*;
- (ii) *Lycopodium*; or
- (iii) *Huberzia*.

3. A recombinant PtIP-65 polypeptide, wherein the amino acid sequence of the PtIP-65 polypeptide has:

- a) at least 80% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Aa as shown in Figures 17a-17k;
- b) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Aa, PtIP-65Ba, PtIP-65Bb, or PtIP-65Ca as shown in Figures 17a-17k;
- c) at least 70% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Fa or PtIP-65Fb as shown in Figures 17a-17k;
- d) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Ha, PtIP-65Gd, or PtIP-65Ge as shown in Figures 17a-17k;
- e) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Hh or PtIP-65Hg as shown in Figures 17a-17k;
- f) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He, or PtIP-65Hf as shown in Figures 17a-17k; or
- g) at least 75% sequence identity, across the entire length of the amino acid sequence, compared to the amino acid sequence PtIP-65Hj or PtIP-65Hk as shown in Figures 17a-17k;

wherein the PtIP-65 polypeptide has insecticidal activity in combination with a PtIP-50 polypeptide as defined in claim 1.

4. The recombinant PtIP-65 polypeptide of claim 3, wherein the PtIP-65 polypeptide:

(A) has one or more characteristics selected from the group consisting of:

- a) a molecular weight of between about 80kDa and about 100kDa;
- b) an isoelectric point between about 4.4 and 5.2; and
- c) does not have chitinase activity;

(B) in combination with a PtIP-50 polypeptide:

- a) specifically binds to brush border membrane vesicles isolated from a Lepidoptera; or
- b) disrupts the ionic balance across brush border membrane vesicles isolated from a Lepidoptera; or

(C) is derived from a species of a fern of the Division Pteridophyta or a primitive plant of the Division Lycopphyta; preferably wherein:

a) the fern is in the Genus:

- (i) *Asplenium*;
- (ii) *Blechnum*;
- (iii) *Bolbitis*.
- (iv) *Nephrolepis*;
- (v) *Polypodium*;
- (vi) *Platyserium*;
- (vii) *Colysis*;
- (viii) *Adiantaceae*; or
- (ix) *Ophioglossum*; or

b) the primitive plant is in the Genus:

- (i) *Selaginella*;

(ii) *Lycopodium*; or

(iii) *Huberzia*.

5 5. A polynucleotide encoding the PtIP-50 polypeptide of claim 1 or 2.

6. A polynucleotide encoding the PtIP-65 polypeptide of claim 3 or 4.

7. A composition comprising the PtIP-50 polypeptide of claim 1 or 2 and the PtIP-65 polypeptide of claim 3 or 4.

10 8. A fusion protein comprising:

a) the PtIP-50 polypeptide of claim 1 or 2;

b) the PtIP-65 polypeptide of claim 3 or 4; or

c) the PtIP-50 polypeptide of claim 1 or 2 and the PtIP-65 polypeptide of claim 3 or 4.

15 9. A method:

a) for controlling an insect pest population, comprising contacting the insect pest population with an insecticidally-effective amount of the PtIP-50 polypeptide of claim 1 or 2 and the PtIP-65 polypeptide of claim 3 or 4;

20 b) of inhibiting growth of or killing an insect pest, comprising contacting the insect pest with a composition comprising an insecticidally-effective amount of the PtIP-50 polypeptide of claim 1 or 2 and the PtIP-65 polypeptide of claim 3 or 4; or

c) of controlling Lepidoptera and/or Coleoptera insect infestation in a transgenic plant and providing insect resistance management, comprising expressing in the plant an insecticidally-effective amount of the PtIP-50 polypeptide of claim 1 or 2 and the PtIP-65 polypeptide of claim 3 or 4;

25 preferably wherein the insect or insect population is resistant to at least one Bt toxin.

30 10. Use of the PtIP-50 polypeptide of claim 1 or 2 and the PtIP-65 polypeptide of claim 3 or 4 to inhibit growth or kill an insect or insect population.

11. A DNA construct comprising:

35 a) the polynucleotide of claim 5; or

b) the polynucleotide claim 5 and the polynucleotide of claim 6;

preferably wherein the DNA construct further comprises at least one heterologous regulatory sequence operably linked to the polynucleotide.

40 12. A transgenic plant or plant cell stably transfected with the DNA construct of claim 11.

13. A method:

45 a) for controlling an insect pest population, comprising contacting the insect pest population with the transgenic plant of claim 12 or

b) of inhibiting growth of or killing an insect pest population, comprising contacting the insect pest population with the transgenic plant of claim 12.

50 Patentansprüche

1. Rekombinantes PtIP-50-Polypeptid, wobei die Aminosäuresequenz des PtIP-50-Polypeptids aufweist:

55 a) wenigstens 80 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-50Aa, wie in Figuren 10a bis 10o gezeigt;

b) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc oder PtIP-50Bd, wie in Figuren 10a bis 10o gezeigt;

EP 3 102 684 B1

c) wenigstens 70 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-50Fb, PtIP-50Fs, PtIP-50Fo, PtIP-50Fk, PtIP-50Fe, PtIP-50Fl, PtIP-50Fn oder PtIP-50Ft, wie in Figuren 10a bis 10o gezeigt;

5 d) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-50Fd, PtIP-50Fm, PtIP-50Fr, PtIP-50Fg, oder PtIP-50Fh, wie in Figuren 10a bis 10o gezeigt;

e) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-50Ga, PtIP-50Gb, PtIP-50Gc oder PtIP-50Gd, wie in Figuren 10a bis 10o gezeigt; oder

10 f) wenigstens 75% Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-50Fa, PtIP-50Ff, PtIP-50Fq, PtIP-50Fi oder PtIP-50Fj, wie in Figuren 10a bis 10o gezeigt;

wobei das PtIP-50-Polypeptid in Kombination mit einem PtIP-65-Polypeptid, wie in Anspruch 3 definiert, eine insektizide Aktivität aufweist.

15 **2.** Rekombinantes PtIP-50-Polypeptid nach Anspruch 1, wobei das PtIP-50-Polypeptid:

(A) ferner eine oder mehrere Eigenschaften umfasst, die aus der Gruppe ausgewählt sind, bestehend aus:

20 a) einem Molekulargewicht von zwischen etwa 80 kDA und etwa 100 kDA;

b) einem isoelektrischen Punkt zwischen etwa 4,4 und 5,2; und

c) es weist keine Chitinase-Aktivität auf;

(B) in Kombination mit einem PtIP-50-Polypeptid:

25 a) es bindet spezifisch an Vesikel der Bürstensaummembran, die aus einem Lepidoptera isoliert werden; oder

b) es stört das ionische Gleichgewicht über die Vesikel der Bürstensaummembran, die aus einem Lepidoptera isoliert werden; oder

30 (C) es wird von einer Spezies eines Farns der Gruppe Pteridophyta oder einer primitiven Pflanze der Gruppe Lycophyta gewonnen;

vorzugsweise wobei:

a) der Fern in der folgenden Gattung ist:

35

(i) *Asplenium*,

(ii) *Blechnum*;

(iii) *Bolbitis*;

(iv) *Nephrolepis*;

40

(v) *Polypodium*;

(vi) *Platyserium*;

(vii) *Colysis*;

(viii) *Adiantaceae*; oder

(ix) *Ophioglossum*; oder

45

b) die primitive Pflanze in der folgenden Gattung ist:

(i) *Selaginella*;

(ii) *Lycopodium*; oder

50

(iii) *Huberia*.

3. Rekombinantes PtIP-65-Polypeptid, wobei die Aminosäuresequenz des PtIP-65-Polypeptid aufweist:

55 a) wenigstens 80 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-65Aa, wie in den Figuren 17a bis 17k gezeigt;

b) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-65Aa, PtIP-65Ba, PtIP-65Bb oder PtIP-65Ca, wie in den Figuren 17a bis 17k gezeigt;

c) wenigstens 70 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der

EP 3 102 684 B1

Aminosäuresequenz PtIP-65Fa oder PtIP-65Fb, wie in den Figuren 17a bis 17k gezeigt;

d) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Ha, PtIP-65Gd oder PtIP-65Ge, wie in den Figuren 17a bis 17k gezeigt;

e) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-65Hh oder PtIP-65Hg, wie in den Figuren 17a bis 17k gezeigt;

f) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He oder PtIP-65Hf, wie in den Figuren 17a bis 17k gezeigt; oder

g) wenigstens 75 % Sequenzidentität über die gesamte Länge der Aminosäuresequenz verglichen mit der Aminosäuresequenz PtIP-65Hj oder PtIP-65Hk, wie in den Figuren 17a bis 17k gezeigt;

wobei das PtIP-65-Polypeptid in Kombination mit einem PtIP-50-Polypeptid, wie in Anspruch 1 definiert, eine insektizide Aktivität aufweist.

4. Rekombinante PtIP-65-Polypeptid nach Anspruch 3, wobei das PtIP-65-Polypeptid:

(A) eine oder mehrere Eigenschaften aufweist, die aus der Gruppe ausgewählt sind, bestehend aus:

- a) einem Molekulargewicht zwischen etwa 80 kDa und etwa 100 kDa;
- b) einem isoelektrischen Punkt zwischen etwa 4,4 und 5,2; und
- c) es weist keine Chitinase-Aktivität auf;

(B) in Kombination mit einem PtIP-50-Polypeptid:

- a) es bindet spezifisch an Vesikel der Bürstensaummembran, die aus einem Lepidoptera isoliert werden; oder
- b) es stört das ionische Gleichgewicht über die Vesikel der Bürstensaummembran, die aus einem Lepidoptera isoliert werden; oder

(C) es wird von einer Spezies eines Farns der Gruppe Pteridophyta oder einer primitiven Pflanze der Gruppe Lycophyta gewonnen; vorzugsweise wobei:

a) der Farn in der folgenden Gattung ist:

- (x) *Asplenium*;
- (xi) *Blechnum*;
- (xii) *Bolbitis*.
- (xiii) *Nephrolepis*;
- (xiv) *Polypodium*;
- (xv) *Platycterium*;
- (xvi) *Colysis*;
- (xvii) *Adiantaceae*; oder
- (xviii) *Ophioglossum*; oder

b) die primitive Pflanze in der folgenden Gattung ist:

- (iv) *Selaginella*;
- (v) *Lycopodium*; oder
- (vi) *Huberia*.

5. Polynukleotid, das für das PtIP-50-Polypeptid nach Anspruch 1 oder 2 codiert.

6. Polynukleotid, das für das PtIP-65-Polypeptid nach Anspruch 3 oder 4 codiert.

7. Zusammensetzung, umfassend das PtIP-50-Polypeptid nach Anspruch 1 oder 2 und das PtIP-65-Polypeptid nach Anspruch 3 oder 4.

8. Fusionsprotein, umfassend:

- a) das PtIP-50-Polypeptid nach Anspruch 1 oder 2;
- b) das PtIP-65-Polypeptid nach Anspruch 3 oder 4; oder
- c) das PtIP-50-Polypeptid nach Anspruch 1 oder 2 und das PtIP-65-Polypeptid nach Anspruch 3 oder 4.

9. Verfahren:

- a) zum Kontrollieren einer Population einer Insektenplage, umfassend ein Inberührungbringen der Population einer Insektenplage mit einer insektizid-wirksamen Menge des PtIP-50-Polypeptids nach Anspruch 1 oder 2 und des PtIP-65-Polypeptids nach Anspruch 3 oder 4;
- b) zum Hemmen eines Wachstums einer Insektenplage oder zum Töten dieser, umfassend das Inberührungbringen der Insektenplage mit einer Zusammensetzung, die eine insektizid-wirksame Menge des PtIP-50-Polypeptids nach Anspruch 1 oder 2 und des PtIP-65-Polypeptids nach Anspruch 3 oder 4 umfasst; oder
- c) zum Kontrollieren eines Lepidoptera- und/oder Coleoptera-Insektenbefalls in einer transgenen Pflanze und zum Bereitstellen einer Insektenresistenzverwaltung, umfassend ein Exprimieren einer insektizid-wirksamen Menge des PtIP-50-Polypeptids nach Anspruch 1 oder 2 und des PtIP-65-Polypeptids nach Anspruch 3 oder 4 in der Pflanze;

vorzugsweise wobei das Insekt oder die Insektenpopulation gegen wenigstens ein Bt-Gift resistent ist.

10. Verwendung des PtIP-50-Polypeptids nach Anspruch 1 oder 2 und des PtIP-65-Polypeptids nach Anspruch 3 oder 4 zum Hemmen eines Wachstums eines Insekts oder einer Insektenpopulation und zum Töten dieses/dieser.

11. DNA-Konstrukt, umfassend:

- a) das Polynukleotid nach Anspruch 5; oder
- b) das Polynukleotid nach Anspruch 5 und das Polynukleotid nach Anspruch 6;

vorzugsweise wobei das DNA-Konstrukt ferner wenigstens eine heterologe Regulatorsequenz umfasst, die mit dem Polynukleotid wirkverbunden ist.

12. Transgene Pflanze oder Pflanzenzelle, die mit dem DNA-Konstrukt nach Anspruch 11 stabil transfiziert ist.

13. Verfahren:

- a) zum Kontrollieren einer Population einer Insektenplage, umfassend das Inberührungbringen der Population der Insektenplage mit der transgenen Pflanze nach Anspruch 12 oder
- b) zum Hemmen eines Wachstums einer Population einer Insektenplage oder zum Töten dieser, umfassend das Inberührungbringen der Population einer Insektenplage mit der transgenen Pflanze nach Anspruch 12.

Revendications

1. Polypeptide PtIP-50 recombinant, dans lequel la séquence d'acides aminés du polypeptide PtIP-50 présente :

- a) au moins 80 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-50Aa telle que représentée dans les figures 10a à 10o ;
- b) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-50Aa, PtIP-50Ba, PtIP-50Bb, PtIP-50Bc ou PtIP-50Bd telle que représentée dans les figures 10a à 10o ;
- c) au moins 70 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-50Fb, PtIP-50Fs, PtIP-50Fo, PtIP-50Fk, PtIP-50Fe, PtIP-50FI, PtIP-50Fn ou PtIP-50Ft telle que représentée dans les figures 10a à 10o ;
- d) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-50Fd, PtIP-50Fm, PtIP-50Fr, PtIP-50Fg ou PtIP-50Fh telle que représentée dans les figures 10a à 10o ;
- e) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en

EP 3 102 684 B1

comparaison de la séquence d'acides aminés PtIP-50Ga, PtIP-50Gb, PtIP-50Gc ou PtIP-50Gd telle que représentée dans les figures 10a à 10o ; ou

f) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-50Fa, PtIP-50Ff, PtIP-50Fg, PtIP- 50Fi ou PtIP-50Fj telle que représentée dans les figures 10a à 10o ;

dans lequel le polypeptide PtIP-50 présentant une activité insecticide en combinaison avec un polypeptide PtIP-65 tel que défini dans la revendication 3.

2. Polypeptide PtIP-50 recombinant de la revendication 1, dans lequel le polypeptide PtIP-50 :

(A) comprend en outre une ou plusieurs caractéristiques choisies dans le groupe constitué par :

a) un poids moléculaire compris entre environ 80 kDa et environ 100 kDa ;

b) un point isoélectrique compris entre environ 4,4 et 5,2 ; et

c) ne présente pas d'activité chitinase ;

(B) en combinaison avec un polypeptide PtIP-65 :

a) se lie spécifiquement à des vésicules membranaires de la bordure en brosse isolées à partir d'un Lepidoptera ; ou

b) perturbe l'équilibre ionique au travers de vésicules membranaires de la bordure en brosse isolées à partir d'un Lepidoptera ; ou

(C) est dérivé d'une espèce de fougère de la division Pteridophyta ou d'une plante primitive de la division Lycophyta ;

de préférence dans lequel :

a) la fougère est dans le genre :

(i) *Asplenium* ;

(ii) *Blechnum* ;

(iii) *Bolbitis* ;

(iv) *Nephrolepis* ;

(v) *Polypodium* ;

(vi) *Platyserium* ;

(vii) *Colysis* ;

(viii) *Adiantaceae* ; ou

(ix) *Ophioglossum* ; ou

b) la plante primitive est dans le genre :

(i) *Selaginella* ;

(ii) *Lycopodium* ; ou

(iii) *Huberia*.

3. Polypeptide PtIP-65 recombinant, dans lequel la séquence d'acides aminés du polypeptide PtIP-65 présente :

a) au moins 80 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65A telle que représentée dans les figures 17a à 17k ;

b) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65Aa, PtIP-65Ba, PtIP-65Bb ou PtIP- 65Ca telle que représentée dans les figures 17a à 17k ;

c) au moins 70 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65Fa ou PtIP-65Fb telle que représentée dans les figures 17a à 17k ;

d) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65Ga, PtIP-65Gb, PtIP-65Gc, PtIP-65Ha, PtIP-65Gd ou

EP 3 102 684 B1

PtIP-65Ge telle que représentée dans les figures 17a à 17k ;

e) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65Hh ou PtIP-65Hg telle que représentée dans les figures 17a à 17k ;

f) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65Hb, PtIP-65Hc, PtIP-65Hd, PtIP-65He ou PtIP-65Hf telle que représentée dans les figures 17a à 17k ; ou

g) au moins 75 % d'identité de séquence, sur l'ensemble de la longueur de la séquence d'acides aminés, en comparaison de la séquence d'acides aminés PtIP-65Hj ou PtIP-65Hk telle que représentée dans les figures 17a à 17k ;

dans lequel le polypeptide PtIP-65 présente une activité insecticide en combinaison avec un polypeptide tel que défini dans la revendication 1.

4. Polypeptide PtIP-65 recombinant de la revendication 3, dans lequel le polypeptide PtIP-65 :

(A) comprend en outre une ou plusieurs caractéristiques choisies dans le groupe constitué par :

a) un poids moléculaire compris entre environ 80 kDa et environ 100 kDa ;

b) un point isoélectrique compris entre environ 4,4 et 5,2 ; et

c) ne présente pas d'activité chitinase ;

(B) en combinaison avec un polypeptide PtIP-50 :

a) se lie spécifiquement à des vésicules membranaires de la bordure en brosse isolées à partir d'un Lepidoptera ; ou

b) perturbe l'équilibre ionique au travers de vésicules membranaires de la bordure en brosse isolées à partir d'un Lepidoptera ; ou

(C) est dérivé d'une espèce de fougère de la division Pteridophyta ou d'une plante primitive de la division Lycophyta ;

de préférence dans lequel :

a) la fougère est dans le genre :

(i) *Asplenium* ;

(ii) *Blechnum* ;

(iii) *Bolbitis* ;

(iv) *Nephrolepis* ;

(v) *Polypodium* ;

(vi) *Platyserium* ;

(vii) *Colysis* ;

(viii) *Adiantaceae* ; ou

(ix) *Ophioglossum* ; ou

b) la plante primitive est dans le genre :

(i) *Selaginella* ;

(ii) *Lycopodium* ; ou

(iii) *Huberia*.

5. Polynucléotide codant le polypeptide PtIP-50 de la revendication 1 ou 2.

6. Polynucléotide codant le polypeptide PtIP-65 de la revendication 3 ou 4.

7. Composition comprenant le polypeptide PtIP-50 de la revendication 1 ou 2 et le polypeptide PtIP-65 de la revendication 3 ou 4.

8. Protéine de fusion comprenant :

- a) le polypeptide PtIP-50 de la revendication 1 ou 2 ;
- b) le polypeptide PtIP-65 de la revendication 3 ou 4 ; ou
- c) le polypeptide PtIP-50 de la revendication 1 ou 2 et le polypeptide PtIP-65 de la revendication 3 ou 4.

9. Procédé :

- a) pour contrôler une population d'insectes nuisibles, comprenant la mise en contact de la population d'insectes nuisibles avec une quantité efficace du point de vue insecticide du polypeptide PtIP-50 de la revendication 1 ou 2 et du polypeptide PtIP-65 de la revendication 3 ou 4 ;
- b) d'inhibition de la croissance ou d'élimination d'un insecte nuisible, comprenant la mise en contact de l'insecte nuisible avec une composition comprenant une quantité efficace du point de vue insecticide du polypeptide PtIP-50 de la revendication 1 ou 2 et du polypeptide PtIP-65 de la revendication 3 ou 4 ; ou
- c) de contrôle d'une infestation par des insectes Lepidoptera et/ou Coleoptera dans une plante transgénique et de fourniture d'une gestion de la résistance des insectes, comprenant l'expression dans la plante d'une quantité efficace du point de vue insecticide du polypeptide PtIP-50 de la revendication 1 ou 2 et du polypeptide PtIP-65 de la revendication 3 ou 4 ;

de préférence dans lequel l'insecte ou la population d'insectes est résistant à au moins une toxine Bt.

10. Utilisation du polypeptide PtIP-50 de la revendication 1 ou 2 et du polypeptide PtIP-65 de la revendication 3 ou 4 pour inhiber la croissance ou éliminer un insecte ou une population d'insectes.

11. Construction d'ADN comprenant :

- a) le polynucléotide de la revendication 5 ; ou
- b) le polynucléotide de la revendication 5 et le polynucléotide de la revendication 6 ;

de préférence dans laquelle la construction d'ADN comprend en outre au moins une séquence de régulation hétérologue reliée opérationnellement au polynucléotide.

12. Plante ou cellule de plante transgénique transfectée de manière stable avec la construction d'ADN de la revendication 11.

13. Procédé :

- a) pour contrôler une population d'insectes nuisibles, comprenant la mise en contact de la population d'insectes nuisibles avec la plante transgénique de la revendication 12, ou
- b) d'inhibition de la croissance ou d'élimination d'une population d'insectes nuisibles, comprenant la mise en contact de la population d'insectes nuisibles avec la plante transgénique de la revendication 12.

Fig. 1

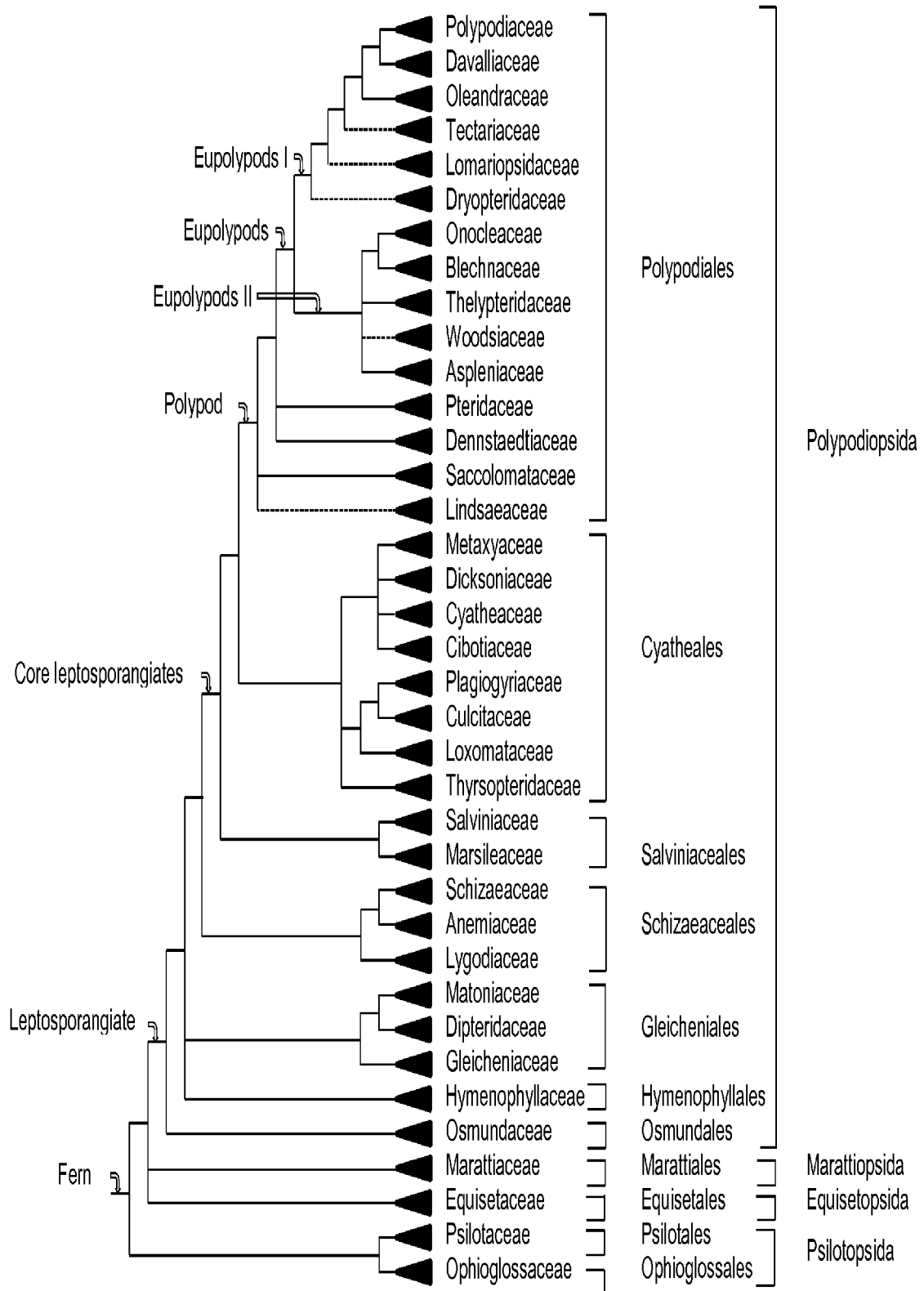


Fig. 2

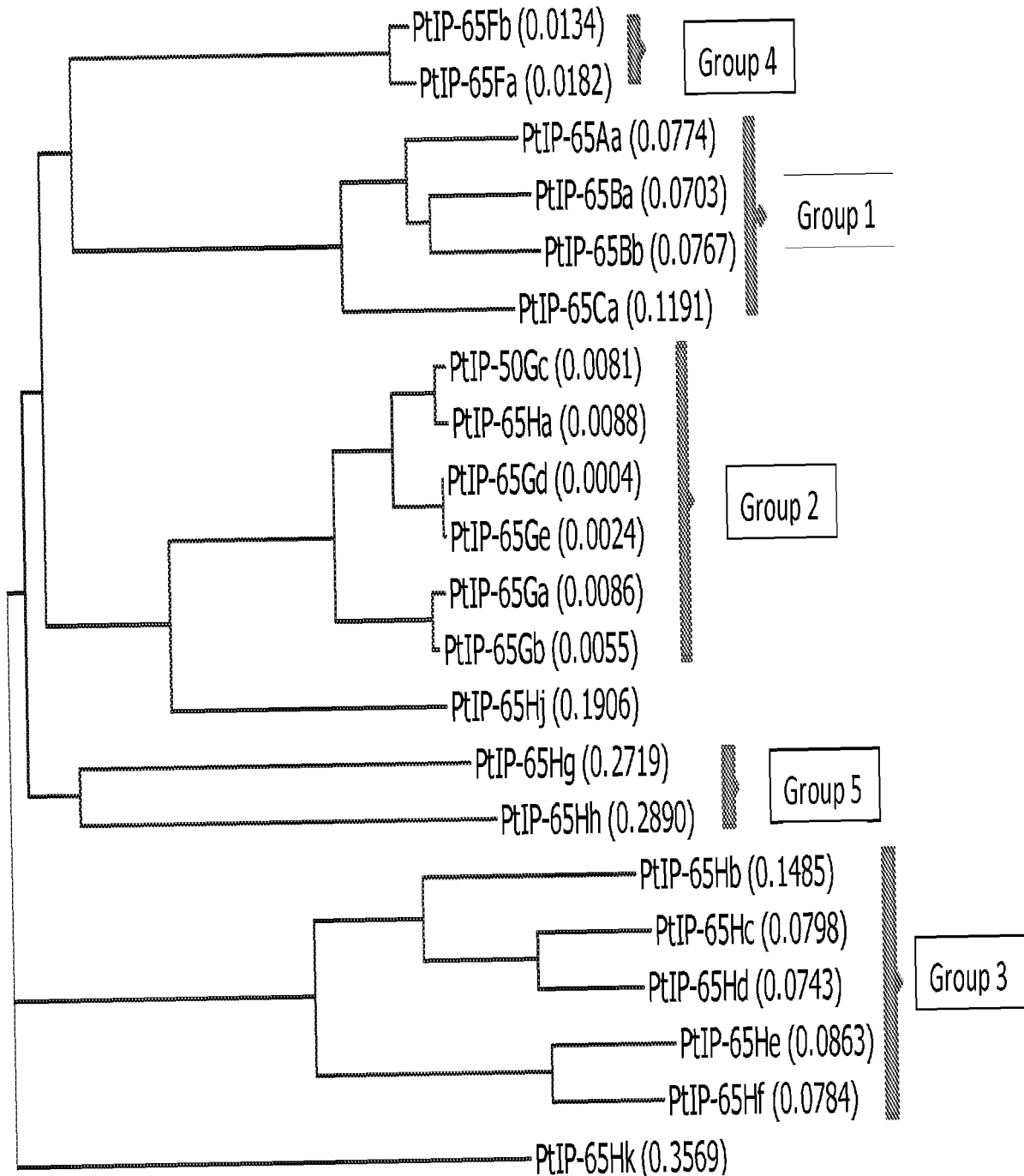


Fig. 3a

	1	60
PtIP-65Aa	(1)	-----
PtIP-65Ba	(1)	-----
PtIP-65Bb	(1)	-----
PtIP-65Ca	(1)	-----
PtIP-65Fa	(1)	-----
PtIP-65Fb	(1)	-----
PtIP-65Ga	(1)	-----
PtIP-65Gb	(1)	-----
PtIP-65Gc	(1)	-----
PtIP-65Ha	(1)	-----
PtIP-65Gd	(1)	-----
PtIP-65Ge	(1)	-----
PtIP-65Hj	(1)	-----
PtIP-65Hg	(1)	MQYGLANTEASPLIEKFQALMEGGIDESILATKLVGAEGDASHLPPPGETPSEDGAGKDP
PtIP-65Hh	(1)	-----MAQLQQHVVNSKHAYGKHAPASKVCEIARAPVHAYKGSN-
PtIP-65Hb	(1)	-----
PtIP-65Hc	(1)	-----
PtIP-65Hd	(1)	-----
PtIP-65He	(1)	-----
PtIP-65Hf	(1)	-----
PtIP-65Hk	(1)	-----

Fig. 3b

	61		120
PtIP-65Aa	(1)	-----MALVIGGLIGGQ-EGYA--FNYYGGTDGRVMQRIKVWA	
PtIP-65Ba	(1)	-----MALVISC PVGGQ-GGSF--FNYYGGTDGRVMQRIKVWA	
PtIP-65Bb	(1)	-----MALVIGWPVGGQ-GGSF--FNYYGGTDGRVMQRIKVWA	
PtIP-65Ca	(1)	-----MALVIGGLIGGG-EGSY--FSYYGGTDGRVMQRIKVWA	
PtIP-65Fa	(1)	-----MAFQTPVTLIGASSEGGQ--FSAYGGTDGKLLKIGVWA	
PtIP-65Fb	(1)	-----MAFQTPVTLIGESSEGGQ--FSAYGGTDGKLLKIGVWA	
PtIP-65Ga	(1)	-----MSLVQTPVYVIGGQ-GGNA--FSYDQSRNGRILRRIGVWA	
PtIP-65Gb	(1)	-----MSLVQTPVYVIGGQ-GGNA--FTYDQSRNGRILRRIGVWA	
PtIP-65Gc	(1)	-----MALYQTPVYVIGGQ-GGSS--FTYDQSRNGRVLTKIGVWA	
PtIP-65Ha	(1)	-----MALYQTPVYVIGGQ-GGSS--FTYDQSRNGKVLKIGVWA	
PtIP-65Gd	(1)	-----MALYQTPVYVIGGQ-GGNS--FTYDQSRNGKVLTKIGVWA	
PtIP-65Ge	(1)	-----MALYQTPVYVIGGQ-GGNS--FTYDQSRNGKVLTKIGVWA	
PtIP-65Hj	(1)	-----MSIHQTPVTLIGGR-GGAA--FTYNAGASGRILRRIGVWA	
PtIP-65Hg	(61)	PNESLETEDVEEHADDSKARSASVTAPLRFIGGP-GGSQ--RSVRGWTNGRVITRMRVYR	
PtIP-65Hh	(40)	-----QG-----DVTAPLTFIGGD-GGKQ--VSKRAWESGKLITRLRVYS	
PtIP-65Hb	(1)	-----MSLTPHLLTASAGG-FIGGDI--FRHSGETDGRVLHRIRLSR	
PtIP-65Hc	(1)	-----MSSLINPHLETIRYGGSFAGGSP--FRILGETEGRVLQRIKISR	
PtIP-65Hd	(1)	-----MSSLINPHLESTYAGSFAGGSP--FRILGETQGRVLQRIKISR	
PtIP-65He	(1)	-----MANLTPLYMQPVQLAGSVNTSLGLWRVSSSETEGKVLKRIKISR	
PtIP-65Hf	(1)	-----MTSLTPPYMQPIQSAGGLFSSF---IVSGETEGKVLQRIKISR	
PtIP-65Hk	(1)	-----MSSGSVIGGVTMVGGPYGSYQ---EVQAWQVGSYISRLTVYV	

Fig. 3c

	121	180
PtIP-65Aa	(36)	ATSRIKAIKVWLSDG---VERTFGDPSRPAGEGLRTSEFRFNSGETVTSLSLWGNAGT
PtIP-65Ba	(36)	AASRIKAIKVWLSDG---VQKTFGDPSRPAGEGLRTSEFSFNTGETVTQLSLWGNAGT
PtIP-65Bb	(36)	ATSRIKAIKVWLSDG---VQKTFGDPSRPSGEGDLETSEFSFKTGETVTQLSLWGNAGT
PtIP-65Ca	(36)	ATSRIKAIKVWLSDG---VQRTEGDPSRPAGEGNLRSAEFSFNTGERVTRLSLWGNIGT
PtIP-65Fa	(38)	GDSRIKAIKVWLTG---EAAQLFGDPHD-PPGEGPLHYKEFAFQPAELITRLSLWGNAGT
PtIP-65Fb	(38)	GDSRIKAIKVWLTG---EAAQLFGDPHD-PPGEGPLLYKEFTFQPAELITRLSLWGNAGT
PtIP-65Ga	(38)	GEWQLRGIRVWMTG---TDTPATFGT-----ATESYSEYTFDGERITRLSLWGNAGT
PtIP-65Gb	(38)	GEWQLRGIRVWMTG---TDTPATFGT-----ATESYSEYTFADGERITRLSLWGNAGT
PtIP-65Gc	(38)	GEWQLRGIRVWMSG---SDTPATFGS-----ASESYSEYTFADGERITRLSLWGNAGT
PtIP-65Ha	(38)	GEWQLRGIRVWMSG---SDSPATFGS-----ASESYSEYTFADGERITRLSLWGNAGT
PtIP-65Gd	(38)	GEWQLRGIRVWMSG---SDSPTFGT-----ASESYSEYTFAGERITRLSLWGNAGT
PtIP-65Ge	(38)	GEWQLRGIRVWMSG---SDSPTFGT-----ASESYSEYTFAGERITRLSLWGNAGT
PtIP-65Hj	(38)	GGSQLRGIRVWWTG---LDSPTVGTGTP-----NVGSYQEFTFQDGERITSLSLWGNAGT
PtIP-65Hg	(118)	ARGTIKAYQIWLTF---DSAPQTHGVP-----GNSDFAEYTFRTGERLTRLTLWGNMGTT
PtIP-65Hh	(77)	GYSKIKAMKVVWFTGDEYSEGTCLGEP-----DGTDYKEYTFSEGERITRMSLWGNNGT
PtIP-65Hb	(41)	EVARISAVEFWLITG---NSTPYVGTGTPR-----ADNSSYEFVDGERITRLDFRTHLFGV
PtIP-65Hc	(43)	MANAIDALEFWLITG---DSTPRVGTGTVR-----SENSSDFSEGERITGLRFRNALFGI
PtIP-65Hd	(43)	IATRIEALEFWLITG---DSTPRVGTGTVR-----SENSSYDFAEGERITRLRFRTRSFRGF
PtIP-65He	(45)	LTRSIPTALEFWLITG---DSNPVCGTGPGR-----AESSTFEFSEGERITTLVLQDAQNIP
PtIP-65Hf	(42)	FSRNIAALEFWLITG---DSNPFLCGTGPGR-----AESSTFEFSEGERITTLVLQEDPQLP
PtIP-65Hk	(40)	ESMRVRCARVWVKG---QSASTTFGD-----ATGSSADFEFETGETITANSLWQGEWEN

Fig. 3d

	181	240
PtIP-65Aa	(93) R-----	SGWIYERTSQNRTFDFGMYSWGKKTEYPQSVASGIWVGIRGR--ASSDIDAL
PtIP-65Ba	(93) R-----	SGWIYERTNQNRITFDFGMYSWGKKTEYPQDVSSGIWVGITGR--AKSDVDAL
PtIP-65Bb	(93) R-----	SGWLYERTSHNRTFDFGMYSWGKKTEYPQNVASGIWVGITGR--ASSDLGL
PtIP-65Ca	(93) R-----	SGWIYFETNQRRFDFGMYSWGKQTEFPQTVASGIWVGFTGR--ASLDVDAL
PtIP-65Fa	(95) R-----	AGWIYFETNQSRFDFGMYSWGKKKEYPVDVASGICAGVMGT--AASDINNI
PtIP-65Fb	(95) R-----	AGWIYFETNRSRFDGMYSWGKKKEYPVDVASGICAGVMGT--AASDINNI
PtIP-65Ga	(89) R-----	SGGIRFYTTTGGSEFFHKMTSWGLQTEYPIDVASGLCVGITGR--ANVDVDSL
PtIP-65Gb	(89) R-----	SGGIRFYTTTGGSEFFHKMTSWGLQTEYPIDVASGLCVGIMGR--ANVDVDSL
PtIP-65Gc	(89) R-----	SGAIRFYTTTGGSEFFPKMTSWGLQTEYPIDVASGLCVGILGR--ANVDVDSL
PtIP-65Ha	(89) R-----	SGAIRFYTTTGGSEFFPKMTSWGLKTEYPMDVASGLCVGILGR--ANVDVDSL
PtIP-65Gd	(89) R-----	SGAIRFYTTTGGSEFFPKMTSWDLKTEYPIDVASGLCVGIIGR--ASADIDSL
PtIP-65Ge	(89) R-----	SGAIRFYTTTGGSEFFPKMTSWDLKTEYPIDVASGLCVGIIGR--ASADIDSL
PtIP-65Hj	(90) R-----	SGGIRFYTTTGRRFFHHMTSWGLKQEYPVDVVDGVCVGLTGR--QGADIDAL
PtIP-65Hg	(169) R-----	AGWIEFETSLGGRFSYGMHWSLRTPYPVDVGGILVGYIFN--AGEDVDAH
PtIP-65Hh	(131) R-----	AGWISLSTNKGGFVSYGMHGWPLCTEYPVNVGSGILAGAIYN--AGCDIDAH
PtIP-65Hb	(92) V-TIQWDNIARVRETSRGRIFEFSSREPSGQWFTANVSGVCGVMSGV-EANGALTRL	
PtIP-65Hc	(94) G-QNQWNHVARVWESTSRGRTFEYGSTREPTGQWFEVNVGSGVCGVAGN-VMLNSLNML	
PtIP-65Hd	(94) GVQTQWDHVARVWESTSRGRTFEYGSTREPSGQWFEANVSGVCGVAGN-VMLDSLNML	
PtIP-65He	(97) -----	THVGRIRFQTSLLRTEYGMVQPTGKVTTVNVGSGVCGVRASHSSTYGISVF
PtIP-65Hf	(94) Y-----	TVVGRIRFQTSRLRTEYGMVQPSGRVITVNVGSGVCGVRAG-TALSVISIL
PtIP-65Hk	(91) P---DHIRAGHLYFTTNGREFSAGPS--TMWSETVIDVQSGWLVGLQAY--TGDDINKW	

Fig. 3e

	241	300
PtIP-65Aa	(144) GVVFLQ-RIQSCRLTSVQYPTLQFSGSSGGTTSIVRTPPKSFNLGNTADQDDPSS-TEQ	
PtIP-65Ba	(144) GVVFLQ-PILSCRLISVDYPTLQFSGTSGGTTSINLTAGKTFNLQNAHQEDPSS-TEK	
PtIP-65Bb	(144) GVVFLR-SIQSCRLINVOYPTLQFSGTAGGTTSITRTTAAKTFNLQNTANQDDPSS-TEQ	
PtIP-65Ca	(144) GVVFLR-PIQSCQLMNVQYPTLQFSGSSGATSITPTASSTKSFTLLNTADHEDQSS-TQQ	
PtIP-65Fa	(146) GFVFLK-PIQSSKLINVOYPSLSFDTQG-----ISPQTLKEFNHTNTS--NNP	
PtIP-65Fb	(146) GFVFLK-PIQSSKLINVOYPSLSFDTQG-----ISPQTLKEFNHTNTS--NNP	
PtIP-65Ga	(140) GVLFLR-TIAPARMINVSYPTLGLAQAG-----IIPVTLDSYNDSNNAG-TIS	
PtIP-65Gb	(140) GVLFLR-TIASARMINVSYPTLGLAQAG-----IIPVTLDSFNDSNNAG-TIS	
PtIP-65Gc	(140) GFLFLR-TIASARMINVSYPTLGLAQAG-----IIPITLDSYNDSNNAG-AIS	
PtIP-65Ha	(140) GFLFLR-TIASARMINVSYPTLGLAQAG-----IIPITLDSYNDSNNAG-AIS	
PtIP-65Gd	(140) GFMFLR-TIASSRMINVSYPTLGLAQAG-----IIPVTLDSYNDSNNAG-SIS	
PtIP-65Ge	(140) GFMFLR-TIASSRMINVSYPTLGLAQAG-----IIPVTLDSYNDSNNAG-SIS	
PtIP-65Hj	(141) GFMFLR-TMTSARMINVKYPTLGLQTAG-----IVPVTLDFMSDSNNAS-SIS	
PtIP-65Hg	(220) GFWFLN-HIEQALTNVRYPTLGFDTAG-----IVPTALDTRFRNNS--STP	
PtIP-65Hh	(182) GNYFLSSSVTSSKLENVKYPTLKFDTSG-----ITPVSLDTYKQNTS--SSP	
PtIP-65Hb	(150) GFMFLR-SIQSVGFSSVEYPTLSTST-----ILTTPILEQLPDTFKSNDD-DEP	
PtIP-65Hc	(152) GFVFLR-SIQSVGFSSVEYPMFSTS-----ITRTSILEQLPDTFKSNDD-DEP	
PtIP-65Hd	(153) GFVFLR-SIQRVGFTSVEYPTISSS-----IARTFILSHLPDTFKSNDD-DEP	
PtIP-65He	(151) GFMFLR-PIQSVRLHGVYPTISSTS-----TITTTILQELPATIKNDND-HEP	
PtIP-65Hf	(148) GFMFLR-PIRSVRLHGVYPTISSIS-----TITTTILQELPATLKNDDD-HEP	
PtIP-65Hk	(144) GFVFLK-PLLVFALADVQYTGLODVG-----AIVPTTLDLDETNNSSSTGSD	

Fig. 3f

	301	360
PtIP-65Aa	(202) LAWQLADEPSFDNVSHSWLSNNTSTGLLQ-----FIATSTVSVQARTPALAVN-----	
PtIP-65Ba	(202) LAWELADEPSFDNVSHFWLSNISTRLLQ-----FISTSTISVQARTPALAVN-----N	
PtIP-65Bb	(202) LAWQLADEPSFDNVSHFWLSNNTSTGLLQ-----FISTSTISVQARTPALTIVN-----	
PtIP-65Ca	(202) LAWELADEPRFDNVFLSWILSNPSAGLLQ-----FITSSISVQARIPALAVN-----	
PtIP-65Fa	(191) TNWEEKGS-SAVTVSSSWSLTIG-----LAVHASVTVVEAGIPAVADV-----	
PtIP-65Fb	(191) TSWEEKGS-SAVTVSSSWSLTIG-----LAVHASVTVVEAGIPAVADV-----	
PtIP-65Ga	(186) KNWTFSGS-RTVTISSSWSLTIG-----LETHASVSVQAGIPMVAEVS-----	
PtIP-65Gb	(186) KNWTFSGS-RTVTISSSWSLTIG-----LETHASVSVQAGIPMVAEVS-----	
PtIP-65Gc	(186) KNWTFSGS-RTVTISSSWLITIG-----LEAHASVTVQAGIPSVAEVS-----	
PtIP-65Ha	(186) KNWTFSGS-RTVTISSSWLITIG-----LEAHASVTVQAGIPSVAEVS-----	
PtIP-65Gd	(186) KNWTFSGS-RTVTISSSWLITIG-----LEAHASVTVQAGIPSVAEVS-----	
PtIP-65Ge	(186) KNWTFSGS-RTVTISSSWLITIG-----LEAHASVTVQAGLPSVAEVS-----	
PtIP-65Hj	(187) KTWSEFGS-REVTVSSSWSTTIG-----LELHASITVSAGIPLVANVE-----	
PtIP-65Hg	(265) RDWDFSRN-MSRSTERFWSITVD-----LTVHASITVSAGFPGIANVS-----	
PtIP-65Hh	(228) RNWSEGGK-RTVKSTTKWGLKIA-----NTFNVELSVEAGVPQVSKSG-----	
PtIP-65Hb	(197) LHVVLGAS-RQLETSTW---TSPAVGLLSHLTGNITVNVSLGINTPTVVPSG-PEGAS	
PtIP-65Hc	(198) LQMVLAGS-RQFKTSSWR-VSSPTVGLLSHLTGNILVDVTLGINTPTVVPTG-LAGAS	
PtIP-65Hd	(199) LQMVLAGS-RQFKTSSWR-AQSPAVGLLSHLTANNITLDLTLGINTPTVVPTG-TAGAS	
PtIP-65He	(198) LHVVLGAS-RQCITSSWRTPADREGLVSHLVGRAISINIDLGIDTPKIVATGGTAGAS	
PtIP-65Hf	(195) LHVVLGAS-RQCFTSSWRTPQAYRAGLVSHLVGSAISINMDLGIDAPKIVATGGTAGAS	
PtIP-65Hk	(190) VNWSLEGS-KAETVSWFSTDS-----LTATIGFEVSAGIPEVAQVK-----	

Fig. 3g

	361	420
PtIP-65Aa	(251) DVVGNQLSASDTQSSSLSSSSSLLLPWSR-----	SGSLLPSK-SFALSALPY-RGN
PtIP-65Ba	(252) IVVGNQLSASDTQSSSLSSSSSLLLPWSR-----	NGSLLPSE-SLTLALPF-GGT
PtIP-65Bb	(251) DVVGNLPSDSDTQSSSLSSSSSLLLPWSR-----	NGSLLPSE-SLTLALPY-DGI
PtIP-65Ca	(251) NVVGNQLSASDTQSSSLSSSSSLLLPWSR-----	SGSLLPSQSLTTLALTY-RGD
PtIP-65Fa	(233) GEFGWEVSASTTSQS--STTEIDTLWGV-----	SGTLSAGE-SIHLKALTR-KGL
PtIP-65Fb	(233) GEFGWEVSASTTSES--STTEIDTLWGV-----	SGTLSAGE-SIHLKALTR-KGL
PtIP-65Ga	(228) GEFGWSVSVSGTYAE--TQEESRRLTNQ-----	SGTLEPGQ-WISLQATTR-RGT
PtIP-65Gb	(228) GEFGWSVSVSGTYAE--TQEESRRLTNQ-----	SGTLEPGQ-WISLQATTR-RGT
PtIP-65Gc	(228) GEFGWSVSVTGSYTS--TQEESRRLTNQ-----	SGTLEPGQ-WISLQATTR-RGT
PtIP-65Ha	(228) GEFGWSVSVTGSYTS--TQEESRRLTNQ-----	SGTLEPGQ-WISLQATTR-RGT
PtIP-65Gd	(228) GEFGWSVSVSGSYTS--TQEESRRLTNQ-----	SGTLEPGQ-WISLQATTR-RGT
PtIP-65Ge	(228) GEFGWSVSVSGSYTS--TQEESRRLTNQ-----	SGTLEPGQ-WISLQATTR-RGT
PtIP-65Hj	(229) GQYGNWAISTSSFTYI--NHSETRFLWQN-----	SGVLEPGQ-WISLQALTR-RGT
PtIP-65Hg	(307) GQYGNWEGVTGHFEE--TETSEHDLWSV-----	GGRVQPGD-VVDLVALTR-TGT
PtIP-65Hh	(270) AKFGWTISVADHEE--SEERTQELVWST-----	GGTLQPGE-TVDLVALTR-QGN
PtIP-65Hb	(252) TTFHWQTVRTFPPSTN-AMEGSIANLTVTNEYSVWCHISDTIAPAQLLP-KHVALVGEGR	
PtIP-65Hc	(255) TTFQWETVRAFPSTN-AIQGSISNLTVSTDEYSVWCHISDTVAPAQSLP-KHAAWVGEGR	
PtIP-65Hd	(256) TAFQWQTVRTFPPSINAEIQGTIANLTVSTAEYSVWCHISDTVAPAQSIP-KHSAWVGEGR	
PtIP-65He	(257) TNFGWETARTFPPSTN-AVEGSIADLTVRTNEYSVWGHVSDTLAPAQSLISRRVLI GEGS	
PtIP-65Hf	(254) TNFGWETARTFPPSTN-AIQGSIADLIVSTNAYSVWGHVSDTLAPAQSLISRRALIGEGR	
PtIP-65Hk	(232) ESFQVQVGTSTTRSS--SYSEEHLTWST-----	GGVLKPGQ-HVVASAVTQ-VGT

Fig. 3h

	421		480
PtIP-65Aa	(300)	VSGLSFNGSARVTTSGG-----TFSFSGLQGRFTGQSYAIDITTQ-----	
PtIP-65Ba	(301)	VSGLPFDGSARVTTTGG-----TFSFSGLHGFYTGQSHVIAITTQ-----	
PtIP-65Bb	(300)	VNGLPFTGSARVTTSHG-----TFSFSGLKGAFTEGSHVIAVTAQ-----	
PtIP-65Ca	(301)	ARNLFFNGSAQVTTTGS-----RFSFPGLQGLFTGQSYVPAITSQ-----	
PtIP-65Fa	(280)	IS-VPYIGSIQVTLKSG-----DVFOYPLKGQYSGISYSGVTVT-----	
PtIP-65Fb	(280)	IS-VPYTGSIQVTLKSG-----DIFQYPLKGQFSGVSYSGVTVT-----	
PtIP-65Ga	(275)	IT-LPFQATMEITLQSG-----TIFQYAISSMYSGVDYTSVDITNTGTRASD-----	
PtIP-65Gb	(275)	IT-LPFQATMEITLQSG-----TIFQYAISSMYSGVDYTSVDITNTGTRASD-----	
PtIP-65Gc	(275)	IT-LPYQGTMEITLQSG-----TVFRYPISSMYSGVDYTSVDITNTGTRALKN-----	
PtIP-65Ha	(275)	IT-LPYQGTMEITLQSG-----TVFRYPISSMYSGVDYTSVDITNTGTRALKN-----	
PtIP-65Gd	(275)	IT-LPYQGTMEITLQSG-----TVFOYPISSMYSGVDYTSVDITNTGTRALK-----	
PtIP-65Ge	(275)	IT-LPYQGTMEITLQSG-----TVFOYPISSMYSGVDYTSVDITNTGTRALK-----	
PtIP-65Hj	(276)	IT-LPYQATMQITLQNG-----TVFTYPIITAQYAGVDYTSVEIVSQGTRDLGSDHLAINK-----	
PtIP-65Hg	(354)	LN-IPYEGTMVVRMRNG-----ASFSAVVRGTYRGLSYTGTKINDNST-----	
PtIP-65Hh	(317)	INDLRREGTMVVTLKNG-----ASFRFPLSGPKGICYTGVEIKDHEETKTMAMKSYASL-----	
PtIP-65Hb	(310)	ITALPCSARIQVLTSSAYDLPFATFSFPVQSLYNGRAHSQVQLIDP-----	
PtIP-65Hc	(313)	ITALPCSANIQVFTSGNNFPFGTFSFPVRLLYDGGAHSNVQVL-----	
PtIP-65Hd	(315)	ITALPCSANIQVITSGNNLPFASFSPVRLFYDGGAHSTVQVL-----	
PtIP-65He	(316)	IDNLQCSARIQVFTDADGGLPFATFTFPVGVLYSARAHSDVQVLS-----	
PtIP-65Hf	(313)	IDNLQCSARIQVFTN-DSALSLATFTFPVGVLYSARAHSDVQVLS-----	
PtIP-65Hk	(279)	LSGLPYTATVVIKTKDAS-----SYSYATSGSYSGISCTEVQLDVKVT-----	

Fig. 3i

	481	540
PtIP-65Aa (340)	-----	-----
PtIP-65Ba (341)	-----	-----
PtIP-65Bb (340)	-----	-----
PtIP-65Ca (342)	-----	-----
PtIP-65Fa (318)	-----	-----
PtIP-65Fb (318)	-----	-----
PtIP-65Ga (321)	-----	-----
PtIP-65Gb (321)	-----	-----
PtIP-65Gc (322)	-----	-----
PtIP-65Ha (322)	-----	-----
PtIP-65Gd (321)	-----	-----
PtIP-65Ge (321)	-----	-----
PtIP-65Hj (330)	DVRYIAAANGAAVGT'TTTNAPPHYVHPIRGAPIVEPVKFSVGATYINDTDNITQEVDTTA	
PtIP-65Hg (396)	-----	-----
PtIP-65Hh (372)	CL-----	-----
PtIP-65Hb (356)	-----	-----
PtIP-65Hc (357)	-----	-----
PtIP-65Hd (359)	-----	-----
PtIP-65He (361)	-----	-----
PtIP-65Hf (357)	-----	-----
PtIP-65Hk (322)	-----	-----

Fig. 4a

	1		50
PtIP-65Aa	(1)	MALVIGGLIGGQGGYAFNYYGGTDGRVMQRIKVAATSRIKAI SVWLSDG	
PtIP-65Ba	(1)	MALVISC PVGGQGGSEFNYYGGTDGRVMQRIKVAASRIKAI SVWLSDG	
PtIP-65Bb	(1)	MALVIGWPVGGQGGSEFNYYGGTDGRVMQRIKVAATSRIKAI SVWLSDG	
PtIP-65Ca	(1)	MALVIGGLIGGGGGSYFSYGGTDGRVMQRIKVAATSRIKAI SVWLSDG	
	51		100
PtIP-65Aa	(51)	VERTFGDPSRPAGEGLRTSEFRFNSGETVTSLSLWNGAGTRSGWIYFR	
PtIP-65Ba	(51)	VQKTFGDPSRPAGEGLRTSEFSFNTGETVTQLSLWNGAGTRSGWIYFR	
PtIP-65Bb	(51)	VQKTFGDPSRPSGEGDLETSEFSFKTGETVTQLSLWNGAGTRSGWLYFR	
PtIP-65Ca	(51)	VQRTFGDPSRPAGEGNLRSAEFSFNTGERVTRL SLWNGIGTRSGWIYFE	
	101		150
PtIP-65Aa	(101)	TSQNRTFDFGMYSWGKKTEYPQSVASGIWVGIRGRASSDIDALGVVFLQR	
PtIP-65Ba	(101)	TNQNRTFDFGMYSWGKKTEYPQDVSSGIWVGITGRAKSDVDALGVVFLQP	
PtIP-65Bb	(101)	TSHNRTFDFGMYSWGKKTEYPQNVASGIWVGITGRASSDL DGLGVVFLRS	
PtIP-65Ca	(101)	TNQGRRFDFGMYSWGKQTEFPQTVASGIWVGFTGRASLDVDALGVVFLRP	
	151		200
PtIP-65Aa	(151)	IQSCRLTSVQYPTLQFSGSSGGTTSIVRTTPTKSFNLGNTADQDDPSSTE	
PtIP-65Ba	(151)	ILSCRLISVDYPTLQFSGTSGGTTSINLTTAGKTFNLQNAHQEDPSSTE	
PtIP-65Bb	(151)	IQSCRLINVQYPTLQFSGTAGGTTSITRTTAAKTFNLQNTANQDDPSSTE	
PtIP-65Ca	(151)	IQSCQLMNVQYPTLQFSGSSGATSITPTASSTKSFTLLNTADHEDQSSTQ	

Fig. 4b

	201		250
PtIP-65Aa	(201)	QLAWQLADEPSFDNVSHSWLSNTSTGLLQFIATSTVSVQARTPALAVVN	
PtIP-65Ba	(201)	KLAWELADEPSFDNVSHTWLSNISTRLLQFI STSTISVQARTPALAVVN	
PtIP-65Bb	(201)	QLAWQLADEPSFDNVSHTWLSNTSTGLLQFI STSTISVQARTPALTVN	
PtIP-65Ca	(201)	QLAWELADEPRFDNVFLSWILSNPSAGLLQFIT TSSISVQARIPALAVVN	
	251		300
PtIP-65Aa	(251)	-DVVGWQLSASDTQSSSLSSSSSLLL PWSRSGSLLPSK-SFALSALPYRG	
PtIP-65Ba	(251)	NIVVGWQLSASDTESSSLSSSSSLLL PWSRNGSLLPSE-SLTL SALPFGG	
PtIP-65Bb	(251)	-DVVGWLPSDSDTQSSSLSSSSSLLL PWSRNGSLLPSE-SLTL SALPYDG	
PtIP-65Ca	(251)	-NVVGWQLSASDTQSSSLSSSSSLSLP WSRSGSLLPSQSLTTLSALTYRG	
	301		342
PtIP-65Aa	(299)	NVSGLSFN SARVTTSGG-TFSFSGLQGRFTGQSYAIDITTQ	
PtIP-65Ba	(300)	TVSGLPFDG SARVTTTGG-TFSFSGLHGFYTGQSHVIAITTQ	
PtIP-65Bb	(299)	IVNGLPFTG SARVTTSHG-TFSFSGLKGAF TGESHVIAVTAQ	
PtIP-65Ca	(300)	DARNLFFNGSAQVTTT TGSRFSEFPGLQGLFTGQSYVPAITSQ	

Fig. 5a

	1	50
PtIP-65Ga	(1) MSLVQTPVYVIGGQGGNAFSYDQSRNGRILRRIGVWAGEWQLRGIRVWMT	
PtIP-65Gb	(1) MSLVQTPVYVIGGQGGNAFTYDQSRNGRILRRIGVWAGEWQLRGIRVWMT	
PtIP-65Gc	(1) MALYQTPVYVIGGQGGSSFTYDQSRNGRVLTKIGVWAGEWQLRGIRVWMS	
PtIP-65Gd	(1) MALYQTPVYVIGGQGGNSFTYDQSRNGKVLTKIGVWAGEWQLRGIRVWMS	
PtIP-65Ge	(1) MALYQTPVYVIGGQGGNSFTYDQSRNGKVLTKIGVWAGEWQLRGIRVWMS	
PtIP-65Ha	(1) MALYQTPVYVIGGQGGSSFTYDQSRNGKVLTKIGVWAGEWQLRGIRVWMS	
	51	100
PtIP-65Ga	(51) GTDTPATFGTATGSYSEYTFDGERITRSLWNGAGTRSGGIRFYTTTG	
PtIP-65Gb	(51) GTDTPATFGTATGSYSEYTFADGERITRSLWNGAGTRSGGIRFYTTTG	
PtIP-65Gc	(51) GSDTPATFGSASGSYSEYTFADGERITRSLWNGAGTRSGAIRFYTTTG	
PtIP-65Gd	(51) GSDSPTTFGTASGSYSEYTFAAGERITRSLWNGAGTRSGAIRFYTTTG	
PtIP-65Ge	(51) GSDSPTTFGTASGSYSEYTFAAGERITRSLWNGAGTRSGAIRFYTTTG	
PtIP-65Ha	(51) GSDSPATFGSASGSYSEYTFADGERITRSLWNGAGTRSGAIRFYTTTG	
	101	150
PtIP-65Ga	(101) GSFFHKMTSWGLQTEYPIDVASGLCVGITGRANVDVDSLGVFLFLRTIAPA	
PtIP-65Gb	(101) GSFFHKMTSWGLQTEYPIDVASGLCVGIMGRANVDVDSLGVFLFLRTIASA	
PtIP-65Gc	(101) GSFFPKMTSWGLQTEYPIDVASGLCVGILGRANVDVDSLGFLEFLRTIASA	
PtIP-65Gd	(101) GSFFPKMTSWDLKTEYPIDVASGLCVGIIGRASADIDSLGFMFLRTIASS	
PtIP-65Ge	(101) GSFFPKMTSWDLKTEYPIDVASGLCVGIIGRASADIDSLGFMFLRTIASS	
PtIP-65Ha	(101) GSFFPKMTSWGLKTEYPMDVASGLCVGILGRANVDVDSLGFLEFLRTIASA	

Fig. 5b

	151		200
PtIP-65Ga	(151)	RMINVSYPTLGLAQAGIIPVTLDSYND	SNNAGTISKNWFSGSRTVTVISS
PtIP-65Gb	(151)	RMINVSYPTLGLAQAGIIPVTLDSFNDS	SNNAGTISKNWFSGSRTVTVISS
PtIP-65Gc	(151)	RMINVSYPTLGLAQAGIIPITLDSYND	SNNAGISKNWFSGSRTVTVISS
PtIP-65Gd	(151)	RMINVSYPTLGLAQAGIIPVTLDSYND	SNNAGSISKNWFSGSRTVTVISS
PtIP-65Ge	(151)	RMINVSYPTLGLAQAGIIPVTLDSYND	SNNAGSISKNWFSGSRTVTVISS
PtIP-65Ha	(151)	RMINVSYPTLGLAQAGIIPITLDSYND	SNNAGISKNWFSGSRTVTVISS
	201		250
PtIP-65Ga	(201)	SWSLTSGIETHASVSVQAGIPMVAEVS	GFEFGWSVSVSGTYATTQEESSL
PtIP-65Gb	(201)	SWSLTSGIETHASVSVQAGIPMVAEVS	GFEFGWSVSVSGTYATTQEESSL
PtIP-65Gc	(201)	SWTLTTGIEAHASVTVQAGIPSVAEVS	GFEFGWSVSVTGSYTSTQEESSL
PtIP-65Gd	(201)	SWTLTSGIEAHASVTVQAGIPSVAEVS	GFEFGWSVSVSGSYTSTQEESSL
PtIP-65Ge	(201)	SWTLTSGIEAHASVTVQAGLPSVAEVS	GFEFGWSVSVSGSYTSTQEESSL
PtIP-65Ha	(201)	SWTLTTGIEAHASVTVQAGIPSVAEVS	GFEFGWSVSVTGSYTSTQEESSL
	251		300
PtIP-65Ga	(251)	TWNQSGTLEPGQWISLQATRRGTITL	PFQATMEITLLSGTIFQYAISSM
PtIP-65Gb	(251)	TWNQSGTLEPGQWISLQATRRGTITL	PFQATMEITLLSGTIFQYAISSM
PtIP-65Gc	(251)	TWNQSGTLEPGQWISIQATRRGTITL	PYQGTMEITLQSGTVFQYPISSM
PtIP-65Gd	(251)	TWNQSGTLEPGQWISIQATRRGTITL	PYQGTMEITLQSGTVFQYPISSM
PtIP-65Ge	(251)	TWNQSGTLEPGQWISIQATRRGTITL	PYQGTMEITLQSGTVFQYPISSM
PtIP-65Ha	(251)	TWNQSGTLEPGQWISIQATRRGTITL	PYQGTMEITLQSGTVFQYPISSM

Fig. 5c

	301	350
PtIP-65Ga	(301) YSGVDYTSVDITNTGTRASD-HVEVEATEQQVQGVKDQSVQPNKEAECT	
PtIP-65Gb	(301) YSGVDYTSVDITNTGTRASD-HVEVEATEQQVQGVKDQSVQPNKEAECT	
PtIP-65Gc	(301) YSGVDYTSVDITNTGTRALKNEVEVEAVDQQSQ-EGDHNVPNKEVQESK	
PtIP-65Gd	(301) YSGVDYTSVDITNTGTRALK-QVEVQATDQQSQ-EGDHNVPDKEVEERK	
PtIP-65Ge	(301) YSGVDYTSVDITNTGTRALK-QVEVQATDQQSQ-EGDHNVPDKEVEERK	
PtIP-65Ha	(301) YSGVDYTSVDITNTGTRALKNEVEVEAVDQQSQ-EGDHNVPNKEVQESK	
	351	
PtIP-65Ga	(350) LLFAE	
PtIP-65Gb	(350) LLFAE	
PtIP-65Gc	(350) LLFIE	
PtIP-65Gd	(349) VLFTE	
PtIP-65Ge	(349) VLFTE	
PtIP-65Ha	(350) VLFIE	

Fig. 6a

	1	50
PtIP-65Hb	(1) M-SLLTPHLLTASAGG-FIGGDI--FRHSGETDGRVLHRIRLSREVARIS	
PtIP-65Hc	(1) MSSLINPHLETIRYGGSFAGGSP--FRILGETEGRVLQRIRISRMANAID	
PtIP-65Hd	(1) MSSLINPHLESITYAGSFAGGSP--FRILGETQGRVLQRIRVSRIATRIE	
PtIP-65He	(1) MANLLTPLYMQPVQLAGSVNTSLGLWRVSSETEGKVLKRIRVYRLTRSIP	
PtIP-65Hf	(1) MTSLLTPPYMQPIQSAGGLFSSF---IVSGETEGKVLQRIRVYRFRSRIA	
	51	100
PtIP-65Hb	(47) AVEFWLTGNSTPYVYGTPR-ADNSSYEFVDGERITRLDFRTHLFGVV-TI	
PtIP-65Hc	(49) AIEFWLTGDSTPRVYGTVR-SENSSFDFSEGERITGLRFRNALFGIG-QN	
PtIP-65Hd	(49) AIEFWLTGDSTPRVYGTVR-SENSSYDFAEGERITRLRFRTSRFGFGVQT	
PtIP-65He	(51) AIEVWLTGDSNPHVCGTPGAESSTFEFSEGERITTLVLQDAQNIP----	
PtIP-65Hf	(48) AIEVWLTGDSNPHLCGTPGRAESSTFEFSEGERITLLELQEDPQLPY---	
	101	150
PtIP-65Hb	(95) QWDNIARVRFSTSRGRIFEFSGSSREPSGQWFTANVGSVGVMSGV-EAN	
PtIP-65Hc	(97) QWNHVARVWFSTSRGRTFEYGSTREPTGQWFEVNVGSVGVVAGN-VML	
PtIP-65Hd	(98) QWDHVARVWFSTSRGRTFEYGSTREPSGQWFEANVGSVGVVAGN-VML	
PtIP-65He	(97) --THVGRIRFQTSLLRTFEYGMSVQPTGKVTTVNVGSVGVVGRASHSST	
PtIP-65Hf	(95) --TVVGRIRFQTSRLRTFEYGMSVQPSGRVITVNVGSVGVVGRAG-TAL	

Fig. 6b

		151		200
PtIP-65Hb	(144)	GALTRLGFMFLRSIQSVGFSSVEYPTLSTSTILTPILEQLPDTFKSNDD		
PtIP-65Hc	(146)	NSLNMLGFVFLRSIQSVGFSSVEYPMFSTS-ITRTSILEQLPDTFKSNDD		
PtIP-65Hd	(147)	DSLNMLGFVFLRSIQRVGFTSVEYPTISSS-IARTFILSHLPDTFKSNDD		
PtIP-65He	(145)	YGISVFGFMFLRPIQSVRLHGLVYPTISSSTITTTTILQELPATIKNDND		
PtIP-65Hf	(142)	SVISILGFMFLRPIRSVRLHGLVYPTISSISTITTTTILQELPATLKNDDD		
		201		250
PtIP-65Hb	(194)	DEPLHVVLGSRQLETTSTW---TSPAVGLLSHLTGN DITVNVSLGINTP		
PtIP-65Hc	(195)	DEPLQMVLAGSRQFKTSSTWR-VSSPTVGLLSHLTGN NILVDVTLGINTP		
PtIP-65Hd	(196)	DEPLQMVLAGSRQFKTTSTWR-AQSPAVGLLSHLTANNITLDLTLGINTP		
PtIP-65He	(195)	HEPLHWVLGSRQCITSSTWRTQPADREGLVSHLVGRAISINIDLGIDTP		
PtIP-65Hf	(192)	HEPLHWVLGSRQCFTSSTWRTQLAYRAGLVSHLVGSAISINMDLGIDAP		
		251		300
PtIP-65Hb	(241)	TVVPSG-PEGASTTFHWQTVRTRFPSSN-AMEGSIANLTVTTNEYSVWCHI		
PtIP-65Hc	(244)	TVVPTG-LAGASTTFQWETVRAFPSTN-AIQGSISNLTVSTDEYSVWCHI		
PtIP-65Hd	(245)	TVVPTG-TAGASTAFQWQTVRTRFPSINAEIQGTIANLTVSTA EYSVWCHI		
PtIP-65He	(245)	KIVATGGTAGASTNFGWETARTFPSTN-AVEGSIADLTVRTNEYSVWGHV		
PtIP-65Hf	(242)	KIVATGGTAGASTNFGWETARTFPSTN-AIQGSIADLIVSTNAYSVWGHV		

Fig. 6c

	301		350
PtIP-65Hb	(289)	SDTIAPAQLLP-KHVALVGEGRITALPCSARIQVLTSSAYDLPFATFSFP	
PtIP-65Hc	(292)	SDTVAPAQSLP-KHAAWVGEGRITALPCSANIQVFTSGGNNFPFGTFSFP	
PtIP-65Hd	(294)	SDTVAPAQSIP-KHSAWVGEGRITALPCSANIQVITSGGNNLPFASFSP	
PtIP-65He	(294)	SDTLAPAQSLISRRAVLIGEGRIDNLQCSARIQVFTDADGGLPFATFTFP	
PtIP-65Hf	(291)	SDTLAPAQSLISRRAALIGEGRIDNLQCSARIQVFTN-DSALSLATFTFP	
	351		368
PtIP-65Hb	(338)	VQSLYNGRAHSQVQIIDP	
PtIP-65Hc	(341)	VRLLYDGGAHSNVQVL--	
PtIP-65Hd	(343)	VRLFYDGGAHSTVQVL--	
PtIP-65He	(344)	VGVLYSARAHSDVQVLS-	
PtIP-65Hf	(340)	VGVLYSARAHSDVQVLS-	

Fig. 7

	1	50
PtIP-65Fa	(1) MAFQTPVTLIGASSGGQQFSAYGGTDGKLEKIGVWAGDSRIKAIKVWLT	
PtIP-65Fb	(1) MAFQTPVTLIGSSGGQQFSAYGGTDGKLEKIGVWAGDSRIKAIKVWLT	
	51	100
PtIP-65Fa	(51) DEAAQLFGDPPGEGPLHYKEFAFQPAELITRSLWGNGAGTRAGWIY	
PtIP-65Fb	(51) DEAAQLFGDPPGEGPLLYKEFTFQPAELITRSLWGNGAGTRAGWIY	
	101	150
PtIP-65Fa	(101) FETNQRSFDFGMYSWGKKKEYPVDVASGICAGVMGTAASDINNIGFVFL	
PtIP-65Fb	(101) FETNRSRSFDFGMYSWGKKKEYPVDVASGICAGVMGTAASDINNIGFVFL	
	151	200
PtIP-65Fa	(151) KPIQSSKLINVQYPSLSFDTQGISPQTLKEFNHTNTSNNPTNWEFKGSSA	
PtIP-65Fb	(151) KPIQSSKLINVQYPSLSFDTQGISPQTLKEFNHTNTSNNPTSWEFKGSSA	
	201	250
PtIP-65Fa	(201) VTVSSSWSLTTGLAVHASVTVEAGIPAVADVSGEFGWEVSASTTSQSSTT	
PtIP-65Fb	(201) VTVSSSWSLTTGLAVHASVTVEAGIPAVADVSGEFGWEVSASTTSESSTT	
	251	300
PtIP-65Fa	(251) ETDTLSWGVSGLSAGESIHLKALTRKGLISVPYIGSIQVTLKSGDVFQY	
PtIP-65Fb	(251) ETDTLSWGVSGLSAGESIHLKALTRKGLISVPYTGSIQVTLKSGDVFQY	
	301	317
PtIP-65Fa	(301) PLKGQYSGISYSGVTVT	
PtIP-65Fb	(301) PLKGQFSGVSYSYSGVTVT	

Fig. 8a

	1	50
PtIP-65Hg	(1)	MQYGLANTEASPLIEKFQALMEGGIDESILATKLVGAEGDASHLPPPGET
PtIP-65Hh	(1)	-----MAQLQQHVVNSKHAYGKHAPASKVCEIARA
	51	100
PtIP-65Hg	(51)	PSEDGAGKDPNPESLETEDVEEHADDSKARSASVTAPLRFIGGPGGSQRS
PtIP-65Hh	(31)	PVHAYKGSN-----QG-----DVTAPLTFIGGDGGKQVS
	101	150
PtIP-65Hg	(101)	VRGWTNGRVITRMRVYRARGTIKAYQIWL---DSAPQTHGVPGNSDFAE
PtIP-65Hh	(60)	KRAWESGKLITRLRVYSGYSCIKAMKVWFTGDEYSEGTCLGEPDGTDYKE
	151	200
PtIP-65Hg	(148)	YTFRTGERLTRLTLWGNGMGTRAGWIEFETSLGGRFSYGMHWSL RTPYP
PtIP-65Hh	(110)	YTFSEGERITRMSLWGNGNGTRAGWISLSTNKGGVFSYGMHGWPLCTEYP
	201	250
PtIP-65Hg	(198)	VDVSGILVGYIFNAGEDVDAHGFWFLN-HIEQAELTNVRYPTLGFDTAG
PtIP-65Hh	(160)	VNVGSGILAGAIYNAGCDIDAHGYFLSSSVTSSKLENVKYPTLKFDTSG

Fig. 8b

		251		300
PtIP-65Hg	(247)	IVPTALDTFRFRNNSSTPRDWF	SRNMSRSTERTWSITVDLTVHASITVS	
PtIP-65Hh	(210)	IIPVSLDITYKQTNTESSSPRNWS	FGGKRTVKSTTKWGLKIANTFNVELSVE	
		301		350
PtIP-65Hg	(297)	AGFPGIANVSGQYGWEIGVTGHFETTETSE	HDLSWSVGGRVQPGDVVDLT	
PtIP-65Hh	(260)	AGVPQVSKSGAKFGWTISV	ASDHEESEERTQELVWSTGGTLQPGETVDLV	
		351		400
PtIP-65Hg	(347)	ALTRTGTLN-IPYEGTMVVRMRNGASFSY	AVRGTYRGLSYTGTKINDNST	
PtIP-65Hh	(310)	ALTRQGNLNDLRFEGTMVVT	LKNGASFRFPLSGPYKGCYTGVEIKDHEE	
		401	414	
PtIP-65Hg	(396)	-----		
PtIP-65Hh	(360)	TKTMAMKSYASLCL		

Fig. 9

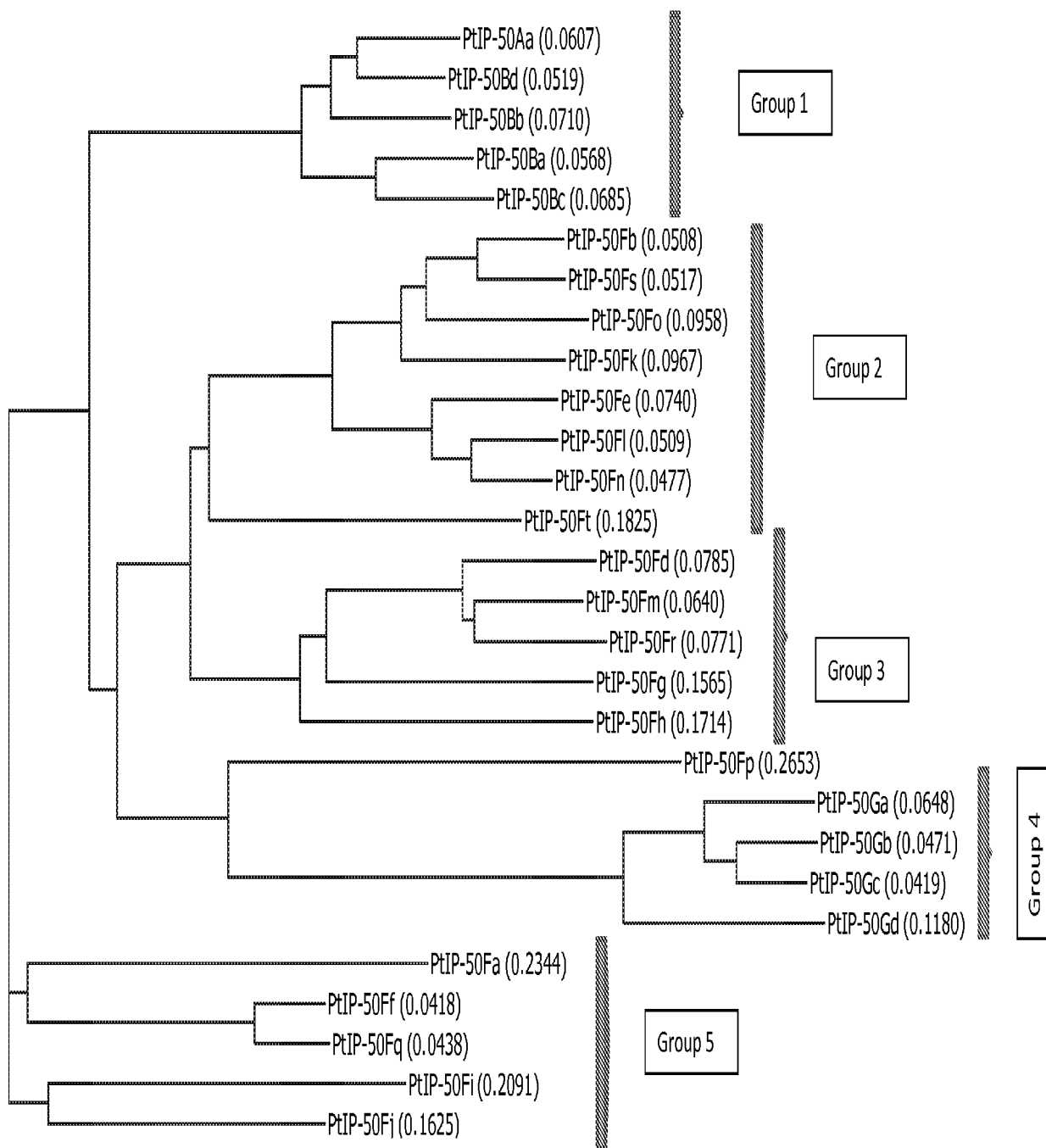


Fig. 10a

	1	70
PtIP-50Aa	(1)	-----MAAGDYSVLYQDVNQIS--IR
PtIP-50Bd	(1)	-----MAGIDYSVLYQDVNQIS--IR
PtIP-50Bb	(1)	-----MADIDYSVLYNDVNGIS--IR
PtIP-50Ba	(1)	-----MADLDYSKLYQDLNQIS--VR
PtIP-50Bc	(1)	-----MADVDSKLYHDLNQIS--IR
PtIP-50Fb	(1)	-----MGGAVPDYSHLYRELNQVSEGMK
PtIP-50Fs	(1)	-----MGGAVPDYSHMYRELNQVSEMR
PtIP-50Fo	(1)	--RERGVLNGLTSDHPFWDGVCSEA-HRSICLVCPDQSLHEIA-MGGAVPDYSHMYRELNQVSEMR
PtIP-50Fk	(1)	EIETEKVLIVCERVAYLFLGGWGACSEAPYRAIGILSADQNPHEIFTMGGAVPDYSHLYRELNQVSEMR
PtIP-50Fe	(1)	-----MERAVPDYSHMYRELNQVSEMR
PtIP-50Fl	(1)	-----
PtIP-50Fn	(1)	-----MERAIPDYSHLYRELNQVSEMR
PtIP-50Ft	(1)	-----MAAAAREYSELYKDLNQVSEMR
PtIP-50Fd	(1)	-----MAAARSAGANEAWREYHDLNQVSDRIR
PtIP-50Fm	(1)	-----MQGRLASMAGSAGAADAWAREYRDLNQVSDRIR
PtIP-50Fr	(1)	-----MAARSAGAADGWAREYRGLNQVSDRIR
PtIP-50Fg	(1)	-----MANDTSSGIVDWRELYRDMNQVSDRIR
PtIP-50Fh	(1)	-----MASITSGAGADWRELYSEMNQVSEMR
PtIP-50Fp	(1)	-----MASLPDYELYKVMNQVSEMR--P
PtIP-50Ga	(1)	-----MHPRAHDGIS-IPLQGGDDHDAEERQISQMRPRFKAN-VVEDYEEIYRGSNSIAREVG
PtIP-50Gb	(1)	-----MHPTGHDGISNIPLEQCHDDYAE--RQMKPRFETNGLELDYERLYAGSNSIAREVG
PtIP-50Gc	(1)	-----MHPTGHDGIS-IPLEQGGDDYAEES-RQMIRPRFKANGLELDYERLYAGSNSIAREVG
PtIP-50Gd	(1)	-----MQQSVHIAARMPGDGEKGRFSSDEEHQMMRPRLTSN-VPEDYEAAYAGSNSIAREVG
PtIP-50Fa	(1)	-----MEYSALYGDVNGVS--LR
PtIP-50Ff	(1)	-----MEYSSLYGDVNGVS--LR
PtIP-50Fq	(1)	-----VAGVS--LR
PtIP-50Fi	(1)	-----MEYSDLYHDVMLA--LP
PtIP-50Fj	(1)	-----MEYSDLYADVNGVS--LR

Fig. 10b

	71	140
PtIP-50Aa	(20)	LEKMDPSEVMVHRMFEVMDLIDVSSGTG--LLSGAEKVERLYVFADVVELP-----SKQRLRPG
PtIP-50Bd	(20)	LEKMDPSEVMVHRMFEVMDLIDVSSGTG--LLAGAEKVERLYVFADVVELP-----SKRRLRPG
PtIP-50Bb	(20)	LERMDPSEVMVHRMFEVMDLIDVSSGTG--VLEGAQNVERLYVFADVVELP-----SKQRLRPG
PtIP-50Ba	(20)	LEKTEPSEVMVHRMFEVMDLIDVSSGSG--LLSGAEKVERLYVFADVVELP-----SKQRLRAG
PtIP-50Bc	(20)	LGNVEPSEVMVHRMFEVLDLIDVSSGSG--LLSGAEKVERLYVFADVVELP-----SKQRLRAG
PtIP-50Fb	(24)	LDQMEPSEVMVIHRMFEIRLPDLIAHRE-----GAEQVERLYVFADVVELSGTS-----LTTLRPG
PtIP-50Fs	(24)	LDHIEPSEVMVIHRMFEIRLPDLIALQE-----GAQQVERLYVFADVVELPETSTSASA-EPVTRRPG
PtIP-50Fo	(67)	LDHMEPSEVMVIHRMFEIRLPDLIALQE-----GAQQVERLYVFADVVELPEMSTSVTVSETVTRRPG
PtIP-50Fk	(71)	LDQMEPSEVMVIHRMFEIRLPDLIAHLE-----GAGQVERLYVFADVVELGGHL-----VTQLRPG
PtIP-50Fe	(24)	MDQMEPSEVMVIHRMFEIRLPDLIAHLD-----GADKVERLYVFADVVELGSVQ-----QTSSLRPG
PtIP-50Fl	(1)	-----MVIHRMFEKLPDLIAHLD-----GADKVERLYVFADVVELGSAG-----QTSNLRPG
PtIP-50Fn	(24)	MDQMEPSEVMVIHRMFEIKFPDLIAHLE-----GADKVERLYVFADVVELGSVR-----QTSSLRPG
PtIP-50Ft	(24)	LDQMEPSEVMVIHRMFEIRLEEDIGHLE-----GAERVERLYVFADVVEVEEGGN--FIETGPLARLPG
PtIP-50Fd	(29)	LDQLEPSEVMVHRMFEIKHSELDIGHLE-----GAEKVERLYVFADVVECDVPPHASSSDGDLVTRRPG
PtIP-50Fm	(34)	LDQLEPSEVMVIHRMFEIKHSELDIGHLE-----GAEKVERLYVFADVVELDVPPHESSSDGDVVTRRPG
PtIP-50Fr	(28)	FDQLEPSEVMVIHRMFEIKHSELDIGHLA-----GAEKVERLYVFADVVECDVPPHENSDDPDLVTRRPG
PtIP-50Fg	(28)	FNQLEPSEVMVIHRMFEIKHSELDIGHLE-----GSEKVERLYVFADVVEYDAN----G-----IARLPG
PtIP-50Fh	(28)	LDQLEPSEVMVIHRMFEIKHSELDIGHLE-----GADKVERLYVFADVVELDNAADPSTP---VDVTRRPG
PtIP-50Fp	(21)	VASRQASEVTSIHRMFEKLNLEVALLT-----KADEVKELVVFADVVEMEG-----GKVELPA
PtIP-50Ga	(57)	VSKVNCSEVMAIHRMFEFRDTSQELLR-----QPERVQLIIVVADVVEIEGSN-----DTSRSLPG
PtIP-50Gb	(56)	LDKVNCSEVMAIHQMEFRDTSQELLR-----RPELVKELIIVVADVVEIEGSN-----ATSRSLPG
PtIP-50Gc	(57)	LNKVICSEVMAIHRMFEFRDTSQELLR-----QPELVRELIVVADVVEIEGAN-----ATSRSLPG
PtIP-50Gd	(58)	VNKVRCGELMAIHQMEFRDTSQELLQ-----QPQLVRELIVVADVVEIEGSR-----GTIVLPG
PtIP-50Fa	(17)	FEKTEPSEVVVHRMEVRLAEINVLGDNNILLQSDRVERLYVLADVVELPP-----I-FSTSMRSLPG
PtIP-50Ff	(17)	FQNMIEPSEVMVHRMEVRLLEEDMTG-----LEGIEKVERLYVLADVVELPSTAT---Q-VFQYERLPA
PtIP-50Fq	(8)	FQNMIEPSEVMVHRMEVRLLEEDMTG-----VEGIEKVERLYVLADVVELPPKAT---S-VFEYERLPA
PtIP-50Fi	(17)	LHRTIELSEVMVHRMEVRLRDLNET-----AVSDKVERLYVLADVVEV-----TLDTVSLPC
PtIP-50Fj	(17)	FQQTEPSEVMVHRMIVALLDIDMTTEPHLQMLAGSEKVERLYVLADVVELP-----A-TATNMRLPA

Fig. 10c

	141		210
PtIP-50Aa	(78)	TDLIVILCRIFVTN-----GRHSTELFASMSMSVAPG-----TG	
PtIP-50Bd	(78)	TDMIILCRIFVFN-----GRHSTELFASMSLNVAAG-----TG	
PtIP-50Bb	(78)	SDMIVILCRIFVRN-----GRHNTELFASMSMSVAAG-----NG	
PtIP-50Ba	(78)	TDMIIVFCRIFVPE-----GRHYAELFASMSMSVADG-----EG	
PtIP-50Bc	(78)	TDMIIVFCRIFVIN-----GHHGTELFASSTMSMVAAG-----EG	
PtIP-50Fb	(80)	TMIVVILCRVLSVRSF-----QSQHGLDFPHRLHAF-PGD-NQMLRIHRRNPDSAQYD	
PtIP-50Fs	(87)	TMIVVILCRVLLFQGS-----YSAYDLDFPHRLHAF-PGE-AQILRIHRRNPDSAQYD	
PtIP-50Fo	(131)	TVIVVILCRVLFYQG-----FARYDLDFPHRLHAFVQPSG-AGVLRMHRLNPDSVQYD	
PtIP-50Fk	(127)	TMIVVILCRVLSLRSF-----TARYGLNDFPHRLHAFVQSA-SRILMHRLIPDSAQYD	
PtIP-50Fe	(80)	SIMVVMCRVLTLEHVDPI-----ATAYQCDFPHRLHAF--FG-SNGVRIDRRN-SDSVHD	
PtIP-50Fl	(48)	SIMVVMCRVLTLEHADWS-----RTSYRFVDFPHRLHAF--FD-LQQARIHRRN-SDSVHD	
PtIP-50Fn	(80)	SIMVVMCRVLTLEHLSA-----AQAYRIDDFPHRLHAF--ND-AQRLRIHRRN-SDSLHD	
PtIP-50Ft	(86)	SIMVILCRVLSVRVLMFDNG-----NNAMRLQDFPHKLRVVEITT-QPTTKLQIHR-ADAEDY	
PtIP-50Fd	(93)	SLSVVFICRVVWHINVGVVQLTS-----TVQMSRTLIVQDFPHRWVFN----ATTGSMHRLHPQTSQEG	
PtIP-50Fm	(98)	SKSVTTFICRVVWHINVALVQISS-----FARISRSVLEDFPHRWVFN----AITGRMHLDPQTLQDG	
PtIP-50Fr	(92)	SVSVTTFICRVVWHVNYGIVQRTS-----TVRVARTVVVQDFPHRLHFN----ATTGSMHRLHPQTSQDG	
PtIP-50Fg	(83)	SISVVFICREWHVSYHLVARMDRNPPRYQLDLAQGVITSDFHMAWHD-----VRRQSMHRLDPETLQEF	
PtIP-50Fh	(89)	SISVVFICRVVWHVSRRLTVGVNSPQYWLPTFDGRPVRIQDFHVKWHMN----TLTRSMHRIDPETLQDL	
PtIP-50Fp	(75)	SRIVTVICRILSLKR-----EDVELGFKFEGPVECEEG--WQVGLNSEMAYIRRTS	
PtIP-50Ga	(113)	SSLVVFICRILIEK-----SKDVVLDLTSWDLTVATTT-----	
PtIP-50Gb	(112)	SSLVVFICRILIEK-----SRNLVLDLGNWNLRAASTT-----	
PtIP-50Gc	(113)	SSLVVFICRILIEK-----SRDVVLDLGNWDLTVATTT-----	
PtIP-50Gd	(114)	SSLVVFICRILIEK-----SKDVVLDLGSWDLTVATSS-----	
PtIP-50Fa	(80)	TVSVVILCRILYLPYLEDASSAS-----G--WAVHAALRLVIVEMHAGFAQVFTIPANDSSAAAP	
PtIP-50Ff	(77)	SISAIILCRVLYIPEVDQRP-----HMAQCSLDFPHRLHVVGAHE--NGGGVMQAFSSDATP	
PtIP-50Fq	(68)	SISAIILCRVLYIPEVQLSGAG-----FRGQCSLDFPHRLHVVGAHE--SGGGVMQAFSSDATA	
PtIP-50Fi	(70)	KVSIVILCRVLYIPEVQSSVLF-----SSLLENMIVRVVLSNN---RMQVYPMATDG--SA	
PtIP-50Fj	(79)	TASVIVILCRVLYVQDVNSPYLA-----CGLHFDLMLPLRVVEDTQQG-RVHVFVVGESASV	

Fig. 10d

	211	280
PtIP-50Aa	(114) SIRGVILSPTTVLTFSS-----	
PtIP-50Bd	(114) SIRGAYLS-TTAFSLSS-----	
PtIP-50Bb	(114) TIRGVNLS-TTMSSSSS-----	
PtIP-50Ba	(115) SIRGVILS-PTVLTFLS-----	
PtIP-50Bc	(114) SIRGVILS-PTTLTFLS-----	
PtIP-50Fb	(132) DYPVVCVHADVLEFVVTG-----GRDIFHLGPLANPCMVTTFPP-----QS	
PtIP-50Fs	(139) DYPVVCVHADVLEFVVTG-----GRDIFHLGPLANPCMVTTHPQ-----LS	
PtIP-50Fo	(183) DYPVVCVLDVVEFVRQR-----PSREFRIGPLAYPSMIFTDERP----PASSI	
PtIP-50Fk	(180) AYPVVCVHADALEFSGTS-----TGGIFHLGPLRYPSMIVTSES-----HS	
PtIP-50Fe	(132) DYPVVTYSANVIEFSGTR-----AETEFALAPLRDPSMIISS-----DLISN	
PtIP-50Fl	(100) DYPVVTYSANVIEFSGTRS-----SSVEFGIGVFLDPSMIVLS-----SDRHS	
PtIP-50Fn	(132) DYPVVTYSANVIEFSGTRS-----LRDIFGIGPLQDPSMIITNT-----ETQPP	
PtIP-50Ft	(143) QYPALCVHADVIQASIRLSSS-----SNRPPMDLRFNFRPNRGSNDNVFSSE-----SRS	
PtIP-50Fd	(152) PDTSCVVAEVVQVAYY-----PDVTFSEFDGSPT-LRFLRLEFESDQLRVTTQT----EGPIWAI	
PtIP-50Fm	(157) MDLCCVVAEVVQIAYY-----PDVTFPESNDSPSFLRFLRLEIVPDQLRVTTQT----DGPVWAL	
PtIP-50Fr	(151) SDTCCVVAEVVQIAYY-----PDVTYPESENDSPVGLRFLRLEFGSDQLRVTRQS----EGPVWAL	
PtIP-50Fg	(148) LEPSFCVVAADDISVAYYSLNGAS-WDPALQFPDTAELAKCALIYSNSNPTPNHLLTSNR-FNGTRWAF	
PtIP-50Fh	(154) SGPLCCVVAIDLRLTSLHPPVGHSNWDAALAFPSMTVLEVEVEPCFDHSTPROGLTLT----SNAAPQW	
PtIP-50Fp	(124) PQVRLSTVAQEVVHNSTG-----AILRVTHPVTVRQRVSPHRP	
PtIP-50Ga	(146) PLHRCSTRAQRVVIASG-----SLSASLLIQVKHKFEWSDGTHLDQ	
PtIP-50Gb	(145) PLHRCSTRAQRVVIASGF-----SLYSSLLIQVKHKFEWSDGTHLDQ	
PtIP-50Gc	(146) PLHRCSTRAQRVVIASG-----SLSASLLIQVKHKFEWSDGTHLDQ	
PtIP-50Gd	(148) PLVRCSTRAQRVVISSG-----SLSASLLIQAKHRLRWSGGSHLDQ	
PtIP-50Fa	(139) ADIGLYTHADRIIYRDD-----PGRSIPVIEVRVGGDSRFSPLP---GSP--EIR	
PtIP-50Ff	(134) SNIGLYTHADRFIYRQAT-----SSASNFVLPDVRVSGSSTFSAPTLPDQWQNLN	
PtIP-50Fq	(128) GNIGLYTHADRFIYREAA-----SPASDFVWPLDVRVSGSSTFSN-TAVPDWQNLN	
PtIP-50Fi	(120) DDVALYTHADRVIRRQG-----AWTLVRVMHMSITGPE-----STFSVPHLPDPAL	
PtIP-50Fj	(135) DKIGLYTHADRVVYRQD-----TSTANPLQPLQLRVIGNGSTFSTEVPRDPWPKTLE	

Fig. 10e

	281	350
PtIP-50Aa	(131) -DALQFKLQSGSMTSVMRLKDVSVAATLTCNVQAAS-----	ASNPLTVKTTGTSPGNICVLGMS
PtIP-50Bd	(130) -NALQFKLQSGSMTSAIQLKDVDLAATLTCDVQAAS-----	ASMPIVVKTTGTSPGNILVLGMS
PtIP-50Bb	(130) -NALQFNLRSGSMTSTVRLKDVDVAAALTCDVQAAS-----	ASNPLTVMTTGTSPGNIWVLGMT
PtIP-50Ba	(131) -NALQFRLECGSMTSVMRLNDVSAATLTCNVQAAS-----	ACVPLKVKTTGTSPGNICVLGLS
PtIP-50Bc	(130) -HALQFKLESGSMTSVMRLNDVSAATLACNVQAAS-----	ASNPLKVKTTGTSPGNICVLGMS
PtIP-50Fb	(173) LEEAPVSSFTGSVRLWTAASSPH----RIDPNNLT-----	FTPGFMVQGGSFNLPFNTQSE----
PtIP-50Fs	(180) LVEAPVSSFTGSVRLWSAATSSPH----SIDPNNLT-----	FTPSFVQGGGSFNLSFATRSE----
PtIP-50Fo	(228) VEPRPVSSFYCTIEFRWSSSNN-----IGPNSVG-----	IGCVFRISGGGS--TFSTGSPRE----
PtIP-50Fk	(221) AVDAQISSFSCNVSLSRSASASSPH----KINPDNVS-----	FQSMFNVTTGG--GFCFAVRPE----
PtIP-50Fe	(174) TVERPASGFFCNVVPNWG-QSPPT----TVDP LRIR-----	FQTGATVVGGSILT--FPLGPE----
PtIP-50Fl	(142) IVERPSSHTLCNVNPVE--QSSPT----MVDPTIR-----	FQSFNWVGSNIMSPQVARAPE----
PtIP-50Fn	(176) VVERPSINSLCTVGLVQGSPPV----TVDPDRIS-----	RHFVWFQSVGGGALG--TLRAAE----
PtIP-50Ft	(194) LIDEPVEIFVYSHIPRFIHALPPEAATVDPASRS-----	NDWYYVSLRRVNSTLSSSVAEAGELEP
PtIP-50Fd	(208) RASLDTTMPDNDGLLNEISAQPA GTIFKNILIPDN-----	LIVSLEGLSLRPNSSLSLTR-VPDNLIP
PtIP-50Fm	(214) RGALEYFMAPDNDGLLNEVSSQPA GTSFVNILIPDN-----	LDVQLFANLRPNSTLTLTR-DPNTIVP
PtIP-50Fr	(208) RGSINYSMPDNDGLMNEVSSQPA GTSFVNILIPDN-----	LNLQMLASLRRNSNSTLTLTR-DPDNIVP
PtIP-50Fg	(216) RFEFS-MYYIDNEDVAKDIALQPPGTRVIFKREGLH-----	LVVLMDSANRIISFPERGDLDAYMP
PtIP-50Fh	(220) VNQLRAAFIFTDQDAMTEILSLPADASVINKNRPS-----	IILTAGQSG-----G--YT-DRNV SAP
PtIP-50Fp	(162) SQYTPGTMKWQINISSAHADLSTQRSHELRTRQRFWS-----	EPERTWLINPQEWS
PtIP-50Ga	(187) YFGRPFSEIASEINSSATATTRTNSAMRSISTWPG-----	TAPVSNFIRVNSEKFNVLVNAV PW
PtIP-50Gb	(187) YFGRPFYFTAIGINSSATITTTASAMRSINTWPG-----	TAPVSNFIRVNSEKFNVLVNAV PW
PtIP-50Gc	(187) YFRRPISFTASRINSSAAITTRTSATRSINTWPG-----	TAPVSNFIQVNSEKFNVLVNAV PW
PtIP-50Gd	(189) YFGRPFSEASSKINSFATTTTQTSYAKQSI STWPG-----	VAPVSNLIVVNSETFNVRVNAV PW
PtIP-50Fa	(185) LSSVQYVREFS-KGQVPAPEDSEIORSDETELIAP-----	SGSEQQLRAAFGASVGMNLLHFHSS
PtIP-50Ff	(186) VSNIQYGPQHLSRGPPLTSSSDSIORSDETELLAQDVWSPLLHVAFSPNAPPGNIPGTQGI FRPSSACS	
PtIP-50Fq	(179) VSSIRYDSQRLSKGPPLTSSSDSIORSDETELLAQDIWSPILHVAFSPNAPPGNVPGTQGLFRPSSACS	
PtIP-50Fi	(166) FNRVMVNVQMD-AGPSIPASEDSIORSDETELVSE-----	RVSNGVVSASIAGSAWMPADNPSATFT
PtIP-50Fj	(186) VSSLLMWTLS-KGQPIPAASEDSIORHDEVELLSPSPD--	RLSVVFAIYAPGNLFSIADVVEYIGAGFC

Fig. 10f

	351	420
PtIP-50Aa	(189) TAVVPES---AVAVITDANILLGMOVTVLI AELVKIANN-EDVLI AAVTRHVEWLNHLLVO-----	
PtIP-50Bd	(188) TAVVIPES---AVAVITDANILLGMOVTVLI AELVKTAANN-EDVVI AAVTRHVEWLNHLLVO-----	
PtIP-50Bb	(188) TAVVIPES---AVAVITDANILLGMOVTVLI AELVKTAANN-EDVII AAVTRHVEWLNHLLVO-----	
PtIP-50Ba	(189) TAAVPES---VAVITDANILLGMOVTVLI AELVKIANN-EDGVIAAVTRHVEWLNHLLVO-----	
PtIP-50Bc	(188) TDAVVPEN---VKAVMVEVNI LGMKLTVLI AELVKIANN-EDAVIAAVTRHVEWLNHLLVO-----	
PtIP-50Fb	(227) DFWLLRRNLP--DALLKDSSTILCMQTSMLIAELVEFSHP-ESDVRAAVTLNAEWLNHLLQASAKS---	
PtIP-50Fs	(234) DFWLLQRNLP--DALLKDSSTILCMQTSMLIAELVEFSHP-ESDVRAAVTLNAEWLNHLLQASAKS---	
PtIP-50Fo	(277) DFWLFRDLP--DAEFKDSSTILCMQTSMLIAELVEFCHP-EPDVRAAVTRSHAKWLNHLLQASVAS---	
PtIP-50Fk	(273) EFWLRRDLP--DALLKDSSTILCMQTSMLIAELVEFSHP-ESDLHAAILNAEWLNHLLQASAAS---	
PtIP-50Fe	(225) GFSILQRNLP--DLLLIDPNILLCMQTSMLIAELVEFSHP-ESDIHAAVTDHVAWLNHLLQASAKA---	
PtIP-50Fl	(194) GFSLFRDLP--DLLLIDPNILLCMQTSMLIAELVEFSHP-ESDIHAAVTENLIWLNHLLQASTKA---	
PtIP-50Fn	(228) GFSLFQDLP--DVLIDPNILLCMQTSMLIAELVEFSHP-ESDIHSAVTENVVWLNHLLQASTKA---	
PtIP-50Ft	(257) AFSLLKPKIP--VVVLTDPNILLAMQTSMLIAELVEVAHP-CAETADAVAKHVEWLNKLLQATKVEG--	
PtIP-50Fd	(270) AFSIFREKAV---ELLIDDPVLSAMQTSMLIGELVEVGQP-EEATTEVYRKHIEWLNHLLQVIEAK---	
PtIP-50Fm	(276) AFSVYKEKEG---ELLIDDPNIPAMQTSMLIGELVEVGQP-ESVTTTEVYRKHIEWLNHLLQVIEAK---	
PtIP-50Fr	(270) AFSVYREKLAV--ELLIDDPNIPPMQTSMLIGELVEVGQP-EEATTEVYRKHIEWLNHLLQVIEAK---	
PtIP-50Fg	(278) AFSLLRQDAKFPIELLIDDPNIPAMQTSMLIGELVEVGNP-ETAVTEVYKHVKWLSKLLHQVIEEK---	
PtIP-50Fh	(275) AFSVLREGAETPIDSLLIDDPNIPAMQTSMLIAELVEVGRP-CAATTEVYKHVEWLNHLLQVIEAK---	
PtIP-50Fp	(213) PETFFLPPGLLPLAILGDSNIMLCMQSTLLIAELVLSYQTHAQATVSAHRHLQWLTNTNLVQVLDLDDSD--	
PtIP-50Ga	(246) KGVVDCQASLPSLDDLEEDVESGIQSTLLIVETILNFQTDNPAIISLARQHAENIVDSLLQVHLP---	
PtIP-50Gb	(246) KGVVDCQALPSLDDLEEDVESGIQSTLLIVETILNFQTDNPAIISLARQHAENIVDSLLQVPLP---	
PtIP-50Gc	(246) KGVVDCQALPSLDDLEEDVESGIQSTLLIVETILNFQTDNPDITVLRQHAENIVDSLLQVPLP---	
PtIP-50Gd	(248) KGVVDCQALPSSDDLEEDVESGIQSTLLIVETILNFQTSNPSITVLAQQAQWIVDSLLQAVHLP---	
PtIP-50Fa	(244) SFDILSLDPV-PAALIDDPNIPTEMQMMMLIAELVLTAAHNPQLIKVVTREVEWLNKMF-----	
PtIP-50Ff	(256) FFHVPPD-V-PANVLTDPSTILGQMNMMLIAELVLAANN-SPQVMNVTKHVEWLNKEL-----	
PtIP-50Fq	(249) FFHVPPD-V-PANVLTDPSTILGQMNMMLIAELVLAANN-SPQVMNVTKHVEWLNKEL-----	
PtIP-50Fi	(228) VFPNPPAQPV-ARGVLTDPYVIIGLQMMMLIAELVQAANN-APLLIRAVTRHVEWLNKEL-----VE	
PtIP-50Fj	(253) YFRIPPADPV-PTVLTDPYVIIGLQMMMLIAELVLAANN-SPPVISVTKHVEWLNKELPDDILALLF	

Fig. 10g

	421	490
PtIP-50Aa	(247)	-AFAAPSEDVVALLYRTQGFINKRNEG-----LIVPRLOYRMYKDLIDRMVQVAQSYDQDFKQKLFVE
PtIP-50Bd	(246)	-AFAAPSDDVVALLYRTQGFINKLQRED-----LIVVFGLOYRMYKDLINRMVQVAQSYDQDFKQKLYVE
PtIP-50Bb	(246)	-AFAAPNEDVITLLYRTQAFINKKREG-----LIVVPRLOYHMYKNLIDRMVQVAQNYDQDFKQKLFVE
PtIP-50Ba	(247)	-AFAAPSEDVVALLYRTQAFINKRKEG-----LIVPRLOYHMYKDLIDRMVQVAQSYDQDFKQKLYVE
PtIP-50Bc	(246)	-ALVAAPSDDVVMLLYRTQALINKQKEG-----LIVPRLOYHMYKDLIDRMVQVAQSYDQDFKQKLYVE
PtIP-50Fb	(291)	--QGTPHHDDYRALIFRAQYVING-IGRSR--GAVVFQLOYDMYSNLINQMARAAADSYDQSLKQQLFIA
PtIP-50Fs	(298)	--EGTPHHADYRALIFRAQYVING-VGKSR--GAVVFQLOYDMYSNLINQVARAAADSYDQSLKQQLFIT
PtIP-50Fo	(341)	--EGTPHHSYDYNALLIFRAQYVING-VGKSR--GAVVFQLOYDMYSNLINQVARAAADSYDQSLKQQLFIA
PtIP-50Fk	(337)	--QGTSHHDDYLALIFRAQYLLING-VGRAR--GAVVFQLOYDMYSNLINQMARAAADTYDQSLKQQLFVA
PtIP-50Fe	(289)	--QGTSHHDDYLALIFRAQYLLING-MGRAR--SLVVFQLOYDVYRNLSQMARVAESYDQSLKQQLFVA
PtIP-50Fl	(258)	--QGTSSYDDYLALIFRSOYLLING-MGRAR--SLVVFQLOYDVYRNLSQMARVAESYDQSLKQQLFLA
PtIP-50Fn	(292)	--QGTSHHDDYLALIFRAQYLLING-MGRAR--SLVVFQLOYDVYRNLSQMARVAESYDQSLKQQLFLA
PtIP-50Ft	(322)	--ILNSSHDEHLALIFRAQYLLMNRPGRTR--NLVVFQLOYDVYSNLVNRMAQVAESYDQSLRQFLFID
PtIP-50Fd	(333)	--QG-QHVEDYVELSFRACQYVINK-VGKIQ--RMVVFQLOYSAYSNLINRMAQVAESYDQALRQFLFIQ
PtIP-50Fm	(339)	--RG-EHVEDYVELSFRACQYVINK-VGRIQ--RLVVFQLOYSAYSNLINRMAQVAESYDQALRQFLFIQ
PtIP-50Fr	(334)	--QG-EPVEDYIELSFRACQYVINK-VGRVQ--RLVVFQLOYSAYSNLINRMAQVAENYDQALRQFLFIQ
PtIP-50Fg	(344)	--NG-KDVEDYIGLSFRACQYVINK-VGRIR--GLVVFQLOYDVYSNLINRMAQVAESYDQALRQFLFIQ
PtIP-50Fh	(341)	--KGGDDVEDYIQLSFRASOYLLINK-VGRID--RFVVFQLOYDYSPLINRMAQVAESYDQALRQFLFIQ
PtIP-50Fp	(281)	--LENEIREQLLALLARAEIASLIPDGSQ--NLIVPRLEYGQYRGLISSMAAVAEAYNSEFTAVNLFIQ
PtIP-50Ga	(313)	--DSTFGVAEVKTLARAOIMLIPDYG--SQHLQVPLAANGFYQEDIDQLLRNAEAYDQFYRETRFVQ
PtIP-50Gb	(313)	--DSTLGVPEAKTLARAOIMLIPDYG--SQHLRVPLAANGFYQEDIERLLRNAEAYDQFYRETRFVQ
PtIP-50Gc	(313)	--NSTLGVPEVKTLARAOIMLIPDYG--SQHLRVPLAANGFYQEDIERLLRNAEAYDQFYRAIRFVQ
PtIP-50Gd	(315)	--NSTTGVPELKTLLANSOMVYIPDYG--SQHLKVPLAVGEFYQEDIDQLLRNAEAYDQFYROIRFVQ
PtIP-50Fa	(303)	-LQLASPNDILALLERVOAFINKMAKQP----HPVVFPRMYSRYEGLINQMVQIAQSYDQDLKQKLFIA
PtIP-50Ff	(313)	-LQVASPNDILALLERVOAFINKMAKEP----RFVVFRLQYHMYGSLINRMVQVAQNYDQEFKQKLFIA
PtIP-50Fq	(306)	-LQVASPNDILALLERVOAFINKMAKQPRFVPRFVVFRLQYHMYGSLINRMVQVAQNYDQEFKQKLFIA
PtIP-50Fi	(288)	ALQVSGNEDLLALLERTETYLMAYES----RSVVFRLQYHMYSDLIHRMVQVAQNYDQEFKQKLFIA
PtIP-50Fj	(321)	RVQLSLPNDILALLERVOAFINKMAKQP----RSVVFRLQYHMYSPLINRMVQVAQNYDQEFKQKLFIV

Fig. 10h

	491	560
PtIP-50Aa	(311) QN---	KILGSYLLEQNKAFAEKKMDAFHSQVIDLRTSSELESTIERMDL SKQMEQNAAMEQAKADMD
PtIP-50Bd	(310) QN---	KILGSYLLEQNKAFAEKKMDAFHSQVIALRTELNNTIERMDL SKQMEQNAAMEQAKADMD
PtIP-50Bb	(310) QN---	KILGSYLLEQNKAFAEKKMDAFHSQVIALRTELNNTIERMGE LSKQMDQNEAMEQAKADMD
PtIP-50Ba	(311) QN---	KILGSYLLEQNKAFAEKKMDASHSQVIALRTELOSTIERMDL SKQMEVQSTAMEKAKADMD
PtIP-50Bc	(310) QN---	KILGSYLLEQNKAFAEKKMDASHSQVIALRTELESTIERMSDL SKQMEVQSKAMKQAKADMD
PtIP-50Fb	(356) QN---	EILGEYLLEQNVEFAAKERDMEVFHSELIAQKTEELQTVMVKIDNLSLQMEQAVEDMEQAKEDME
PtIP-50Fs	(363) QN---	EILGDYLLEQNVEFAAKERDMEVFHSELIAQKTEELQTVMAKIDNLSLQMETQAADMEQAKEDME
PtIP-50Fo	(406) QN---	EILGEYLLEQNVEFAAKERDMEVFHSELISQKETEELRTVAKIDNLSLQMETQVADMEQAKEDME
PtIP-50Fk	(402) QN---	KILGGYLLEQNRAFAAKERDMEVFHSELIAQKTEELQTVMVKIDKLSLQMDTQVADMEQAKEDME
PtIP-50Fe	(354) QN---	KILGGYLLEQNRAFAAKERDMEVFHSELIAQKELELQNTVMVMEQISSQMETQIADMEQAKEDME
PtIP-50Fl	(323) QN---	KVILGGYLLEQNRAFAAKERDMEVFHSELIAQKELELQNTVMFMEQISSQMETQIADMEQAKEDME
PtIP-50Fn	(357) QN---	KILGGYLLEQNRAFAAKERDMEVFHSELIAQKELELQNTVMFMEQISSQMETQIADMEQAKEDME
PtIP-50Ft	(388) QN---	KILGSYLLEQNKAFAEKKMDVVFHSELISQKIEIDNTEILKMDQSLQMESQREDMEQAKEDME
PtIP-50Fd	(397) QN---	KILGGFLEQNRAFAEKQMDVVFYSELITQKQIEIDNTEILQKMKHLGTQMDTQADMEQAKEDME
PtIP-50Fm	(403) QN---	KILGGFLEQNRAFAEKQMDVVFYSELIAQKQIEIDNTEILQKMKHLGAQMETQADMEQAKEDME
PtIP-50Fr	(398) QN---	KILGGFLEQNRAFAEKQMDVVFYSELITQKQIEIDNTEILQKMKHLGAQMETQTAGMEQAKEDME
PtIP-50Fg	(408) NN---	KILGGFLEQNRAFAEKKMDVVFYSELITQKQIEIDNTEILQKMKRSLQMETQADMEQAKEDME
PtIP-50Fh	(406) QTQQNKILGAFLLDQNRFAEKKMDIFYSSELIAQKQIEIDNTEILQKMEQSLQMETQADMEQAKEDME	
PtIP-50Fp	(347) QN---	EILGSYLLEQNKAFAEKKMDAFHGLVVERKQELSSAQETMEELNVOLTQTEADMEQAKEDME
PtIP-50Ga	(379) QV---	EILGSEFLQLSKSLAQKREDIETEFELVIRKQSELDQAIRMNSLMTEIERRSFEADAKARME
PtIP-50Gb	(379) LV---	EILGSEFLQLSKSLAQKREDIETEFELVIRKQSELDQAIRMNSLMPEIERRSFEADAKARME
PtIP-50Gc	(379) LV---	EILGSEFLQLSKSLAQKREDIETEFELVIRKQSELDQAIRMNSLMTEIERRSFEADAKARME
PtIP-50Gd	(381) QV---	QILGSEFLQLSMSLAQKREDIETEFQSLVIRKQSELDQTMRRIDSLMREIERRSFEADAKARME
PtIP-50Fa	(368) QN---	EILGSYLLEQNKAFAEKKMSSEFHLQVSDLRSENDAIQKMTGLGEEMEVEKEADQAYKME
PtIP-50Ff	(378) QN---	KILGSYLLEQNRAFAEKREMSAFHSQVSMRRSELQSAIQNMDNLSLQMESESEAMNEAQENNV
PtIP-50Fq	(375) QN---	EILGSYLLEQNKAFAEKREMSAFHSQVSMRRSELTTALETNMQSLQMETESEAMNEAQENNV
PtIP-50Fi	(354) QN---	EILGSYLLEQNKAFAEKREMSAFHSQVSLRREEDNCLQRMQQLNVQMERENKAMEAQEKMN
PtIP-50Fj	(387) QN---	QILGSYLLEQNKAFAEKREMSAFHSQVSLRREEDNCLQRMQQLNVQMERENKAMEAQEKMN

Fig. 10i

	561		630
PtIP-50Aa	(378)	AG-LIAYONKQVANAVFAVLGAIASIGLAFATGG---ATAPGAVASAGAAVTAAGKAAEG-----	LKKVV
PtIP-50Bd	(377)	AG-LIEYONKQVANAVFAVLGAIASIGLAFATGG---ATAPGAVAAAGTAVTAAGKAAEG-----	LKKVV
PtIP-50Bb	(377)	AG-LIEYONROVANALFAVLGAIASIGLAFATGG---ATAPGAVSAAGAAVTAAGKAAEG-----	LKKVV
PtIP-50Ba	(378)	AG-LIVYONKQVADAVFAVMEIASIGLAFATGG---ATAPGAVASAGAAVSAAGKAGEG-----	LKKVV
PtIP-50Bc	(377)	AG-LVEYQDKQVADAVFAVLEAVASIGLAVATGG---ASAPAVASAGAAVSAAGKAAEG-----	LKKVV
PtIP-50Fb	(423)	AG-LRRFRNQVANAMFAVFRATGAVALTVLTGG---AAAPLAISAAGAVSIAGQAARG-----	LERVL
PtIP-50Fs	(430)	AG-LRRFRNQVANAMFAVFRATGAVALTVLTGG---AAAPLAISTAKGAVSIAGQAARG-----	LQKVL
PtIP-50Fo	(473)	AG-LRRFRNQVANAMFAVFRATAAVALTVVTGG---AAAPLAMSTAKGAVSAAGQAARG-----	LERVL
PtIP-50Fk	(469)	AG-LRRFRDROVANALFAVFRATGAVALTVLTGG---AAAPLAISAAGAVSIAGQAARG-----	LERVL
PtIP-50Fe	(421)	AG-LRRFQNRQVANAMFAVFRATGATALTVVTAG---AAAPAAAMAAKGAVTTAGQAARG-----	LVRVL
PtIP-50Fl	(390)	AG-LRRFQNRQVARAMFAVFRATGAVALTVVTGG---AAAPAAAMSAAGAVSIAGQAARG-----	LERVL
PtIP-50Fn	(424)	AG-LRRFQNRQVARAMFAVFRATGAVALTVVTGG---AAAPAAAMTAAGAVSIAGQAARG-----	LERVL
PtIP-50Ft	(455)	AG-LREFQNRQVANAMFAVIGATAAIGLAFITGG---ATAPAAVGAAGTAVTAAGAAARA-----	LERVV
PtIP-50Fd	(464)	AG-VRKQNEQVARGLFAVLGATAVGLTFLITGG---AAAPLAISTARRAVSVAGAVAQG-----	LQTVL
PtIP-50Fm	(470)	AG-IREFQNRQVARGLFAVLGATAAVGLTFLITGG---AAAPLAMSARSAVSLAGAVAQG-----	LQKVL
PtIP-50Fr	(465)	AAGIRRFQNAQVARGLFAVLGATAAVGLTFLITGG---AAAPLAVSAARRVVTVAGAVAQG-----	LQKVI
PtIP-50Fg	(475)	AG-LRRFQNRQARALFAVLGATAAVGLAFITGG---ATAPAAALGAARTAVTVAGSVVSG-----	LQKVL
PtIP-50Fh	(476)	AG-LRRFQNERVARAVFGVLGATAAVALAFVTGG---ATAGAAI GAARTAVTLAGAARG-----	LQQVL
PtIP-50Fp	(414)	AG-LKKYRDAQARAFFAVMKEVLEIGAAITITGG---ATMGLAVQGGINAVKAVSSLAGK-----	LDMVL
PtIP-50Ga	(446)	QG-LEDYHRRQLTRATEGILGALLQLCASEKFEQGG-ADTAGGIATTVPAAIDMIHAVEDASANTKLAIS	
PtIP-50Gb	(446)	EG-LEDYHRRQLTRATEGILGALLQLCASEKFEQGG-ADTAGGIATTVPAAIDLIHAVQDTSASTKLAIS	
PtIP-50Gc	(446)	EG-LQDYNRRQLTRATEGILGALLQLCASEKFEQGG-ADTAGGIATTVPAAIDLIHAVQDSSASTKLAIS	
PtIP-50Gd	(448)	EG-VLDNYRQVNRAVFGMLGALLQLFASIKFEQGG-ADTAGMETTVSAAIDLIHAVEDASASSKLPMT	
PtIP-50Fa	(435)	QG-LQENKROIVTAVEAVESATSLCALGNVTGGATVAAVGAVANAQAQAVSAVSLANK-----	LQKVM
PtIP-50Ff	(445)	EA-IQENKLLARALFVIGAIASVALAFATGG---ATAPGAVAAAGGAVAAAGRLAAG-----	LQKVV
PtIP-50Fq	(442)	EA-IQENKLLARALFVIGAIASVALAVATGG---ATAPGAVAAARGAVTAAGRLAQQ-----	LQKVV
PtIP-50Fi	(421)	EA-LEARQRRGLARALFAVLEATAAVALTVATGG---AAAPAAVAAAGQAVTAAGTLAQQ-----	LKTVV
PtIP-50Fj	(454)	QA-IAENKQLANALFAVLGAIASVALAFATAG---ATAPGAVAAAGAAVSAAGRLAEG-----	LKKVV

Fig. 10j

	631	700
PtIP-50Aa	(439)	EILEGLQAVMEVVAIKELVCSLQEIIGQLVDAPEMPDLPSDAEWEIFVNEVEAVAEQMPTEVT-EVPANK
PtIP-50Bd	(438)	EILEGLQAVMEVVAIKELVCSLQEIIGQLVNAPEMPDLPSDAEWEIFVNEVEAVAEQMPTEVT-EVPANK
PtIP-50Bb	(438)	EILEGLQVMEVVAIKELVCSLQEIIGQLVDAPEMPDLPSDAEWEIFVNEVEAVAEQMPTEVT-QVAANK
PtIP-50Ba	(439)	EILEGLQAIMEVIAAIKGLVCSLQKIGQLVNAPEMPDLPSDAEWEIFVNEVEAVAEQMPTEVT-QVPANT
PtIP-50Bc	(438)	EVLEGLKAVMEVIAAIKGLVCSLQKIGQLVSAPEMPDLPSDAKWEIFVNEVEAVAEQMPKEVS-QVPANT
PtIP-50Fb	(484)	RILDDLQAAAMELLKTIKDLVESLQEIIGQLVDAPEMPDMPTEADWEIFVNEIEGVAEQMPPEEVS-EVSANK
PtIP-50Fs	(491)	QILDDLQAAAMELLKTIKDLVESLQEVGQLVDAPEMPDMPTEADWEIFVNEIEGVAEQMPPEEVS-EVSANK
PtIP-50Fo	(534)	QILDDLQAAAMELLKTIKDLLESLQAVGQLVDAPEMPDMPTEADWEIFVNEIEGVAEQMPPEEVS-EVSANK
PtIP-50Fk	(530)	QILDDLQAAAMELLKTIKDLVESLQEVGQLVDAPEMPDMPTEADWEIFVNEIEGVAEQMPPEEVS-EVSANK
PtIP-50Fe	(482)	EILDDLQAAAMEVFKTIKDLVESLREVGQLVDAPEMPDMPTEADWSIFVNEIEGVAEQMPPEEVS-EVSANK
PtIP-50Fl	(451)	EILDNLQAAAMEVFKTIKDLVESLREVGQLVDAPEMPDMPTEADWSIFVNEVEAVAEQMPPEEVS-EVSANK
PtIP-50Fn	(485)	EILDNLQAAAMEVFKTIKDLVESLREVGQLVDAPEMPDMPTEADWSIFVNEVEAVAEQMPPEEVS-EVSANK
PtIP-50Ft	(516)	EILDSLQAVMEIVSIIKELVCSLQEIIGQLVEAPEMPDMPTEADWEIFENEIEGVAEQMPTEVS-EVSANK
PtIP-50Fd	(525)	DILEGLQAVMEIVHLINDLISALQELGQPVLEPEMADMPTEADWLIFFVNEVEGVAEQMPTEVS-EVVANK
PtIP-50Fm	(531)	DILEGLQVMEIVVELINDLISALQELGQPVLEPEMAEMPTADWLIFFVNEVEGVAEQMPTEVS-EVVANK
PtIP-50Fr	(527)	DILEGLQVMEIVVELINDLISALQDLGQPVLEPEMAELPQADWLIFFVNEVEGVAEQMPTEVS-EVVANK
PtIP-50Fg	(536)	EVLEGLQAVMEIVVLIKDLFSAIQDLTQAVDLEDMPEMPLQSDWLIFFVNEVEAVAQGMPTES-EVVANK
PtIP-50Fh	(537)	EILDLQAVMEIVGMKELFESLQELGQAVDLEPEMPEMLESWHLIFVNEVEAVAEQMPTEVS-EVVANK
PtIP-50Fp	(475)	QIMEGMEKVMVNLNAIDDLVAVSEINKMVEAPEMPSMPSTHEWDIFENEIEEVAESMPPEEVT-EARTWR
PtIP-50Ga	(514)	QSLMLEKIVDVVNAVNALVESATELEDITHAPELPLIE-PYTWDIENDEIEEFAALMPESEVS-EVVTWK
PtIP-50Gb	(514)	QTLMLEKIVDVVNAVNTLAESATELEDITHAPELPLIE-PYTWDIENDEIEEFAALMPESEVS-EVVTWK
PtIP-50Gc	(514)	QTLMLEKIVDVVNAVVELVESATELEDITHAPELPIIL-PYTWDIENDEIEEFAALMPESEVS-EVVTWK
PtIP-50Gd	(516)	KNLIDLEKMEVNVNAVVELVENATELEDITHAPELPIIS-SYTWDIENDEIDELALMPESEVS-EVVTWK
PtIP-50Fa	(499)	GILEIINQVQTAATAIKVEVLELFDNMGQLLEAPEMPEMPSNYDWLIFFVNEVEAMAEQLEPVEVN-ERIVWK
PtIP-50Ff	(506)	DILQGLQAVMEVVAIROIVESLKNMGQLVEAPEMPEMPTADWLIFFVNEVEAVAEQMPTEVA-EVPVWK
PtIP-50Fq	(503)	DILQGLQAVMEVVAIROIVESLKNMGQLVEAPEMPEMPTADWLIFFVNEVEAVAEQMPTEVA-EVPVWK
PtIP-50Fi	(482)	EILEGLQALMDLVVITRELVENLQITIGQLIDAPEMQEMPSNADWLIFFVNEVEAVAEQMPPEEVFGSAVWK
PtIP-50Fj	(515)	EILEGLAAVMEIVVAIROIVESLQNLGQLVETPEMPDMPSEADWLIFFVNEVEAVAEQMPAEVVGSAVWK

Fig. 10k

	701	770
PtIP-50Aa	(508)	AKCKNVAALGREMSYMAAHIAELQFEIQVQEMLRREIAKQADRLSSIKPADLTN-YLEMVSEMDMRTTRM
PtIP-50Bd	(507)	AKCKNVAALGREMSYMAAHISELQFEIKVQEMLRREIAQKQADRLSSIKPADLTN-YLEMVSEMDMRTTRM
PtIP-50Bb	(507)	AKCKNVAALGREMSYMAAHIAELQYQIQVQEMLRREIAQKQADRLSSISPADLTN-YLEMVSEMDMRTTRM
PtIP-50Ba	(508)	AKCKNVAALGREMSYTAAHIAELQYEQIQVQGMLOQIAKKQADRLSSIKPADLTN-YFEMVSEMDMRTTRM
PtIP-50Bc	(507)	AKCKNVAALGREMSYTAHVHISELQYEQIQVQGMLOQEIAKKQADRLSSIKPADLTN-YLEMVSEMDMRTTRM
PtIP-50Fb	(553)	TSCKNVAAVGRELMTTAYMSQLQYDIQVQAMLOGIASKQADRLSSIQAADLSS-FTEMVTQMDMRTTRL
PtIP-50Fs	(560)	TSCKNVAAVGRELMTTAYMSQLQYDIQVQAMLODIASKQADRLSSIQAADLSS-FTEMVTQMDMRTTRL
PtIP-50Fo	(603)	TSSKNVAAVGRELMTTAYMSQLQYDIQVQAMLODIASKQADRLSSIQAADLSS-FTEMVTQMDMRTTRL
PtIP-50Fk	(599)	TSCKNVAAVGRELMTTAYMSQLQYDVQVQAMLODIARKQADRLSSIQAVDLSS-FTEMVTQMDMRTTRL
PtIP-50Fe	(551)	TSCKNVAAVGRELMTTAYMSQLQYDIKVQAMLODIANKQADRLSSIQAADLSS-FTEMVTQMDMRTTRL
PtIP-50Fl	(520)	TSCKNVAAVGRELMTTAYMSQLQYDIKVQAMLODIANKQADRLSSIQAADLSS-FTEMVTQMDMRTTRL
PtIP-50Fn	(554)	TSCKNVAAVGRELMTTAYMSQLQYDIKVQAMLODIASQADRLSSIQAADLSS-FTEMVTQMDMRTTRL
PtIP-50Ft	(585)	TKCKNVAVLGREMTTAYHISELQYDIKVQGMLOQEIATSEANRLSSIQADLSN-YTEMVTQMDMRTTRM
PtIP-50Fd	(594)	TKCKNVAVLGREMTTAAAYISQLQYDIEMQHMLOQIARKQADRLSAIQLPDLRN-YAELVTQMDMRTTRL
PtIP-50Fm	(600)	TKCKNVAVLGREMTTAAAYISQLQYDINMQHMLOQIARKQADRLSAIQLPDLRN-YGELVTQMDMRTTRL
PtIP-50Fr	(596)	TKCKNVAVLGREMTTAAAYISQLQYDIQMQHMLOQIARKQADRLSAIQLPDLKN-YAELVTQMDMRTTRL
PtIP-50Fg	(605)	TKCKNVAVLGQEMTTAAAYISQLQYDIMQDMLOQIARRQADRLSAIQLPDLTN-FTEMVIQMDMRTTRI
PtIP-50Fh	(606)	TKCKNVAVLGREMTTAAAYISQLQYDIQMQDMLOQIARKQADRLSAIQLSIPNTGTYEMLMQMDMRTTRL
PtIP-50Fp	(544)	TKCRNVAAVCRAISYTAASYIGQLQYELFVSNQELIARRQAEERLEAIQPADLTN-SLEMATQMDMRTSRM
PtIP-50Ga	(582)	AKCRNVAVCREICIAASEACEVQYELFVHARQEQEMARRQAEERLEGMQIADLSSYIELATQADMRTTRL
PtIP-50Gb	(582)	AKCRNVAVCREICIAASEACEVQYELFVHARQEQEMARRQAEERLEGMQIADLSSYIELATQADMRTARL
PtIP-50Gc	(582)	AKCRNVAVCREICIAASEACEVQYELFVHARQEQEMARRQAEERLEGMQIADLSSYIELATQADMRTTRL
PtIP-50Gd	(584)	AKCRNVAVCREICIAANEAEVQYELFVHARQEQEMARRQAEERLEGMQLATDLSSYIELATQADMRTTRL
PtIP-50Fa	(568)	AKCKNVAVLGQEMCTTASHLADLQYQIKVVEMLREIAQSQAERLEGISSADLSS-YTEMVSEMDMRTTRM
PtIP-50Ff	(575)	AKCKNVAVLGQAMCTTAAAYISELQYQITVEEMLQEIQAQADRLVGSAAADLSS-YTEMASQMDMRTTRI
PtIP-50Fq	(572)	AKCKNVAVLGQAMCTTAAAYISELQYQITVEEMLQEIQAQADRLVGSAAADLSS-YTEMASQMDMRTTRI
PtIP-50Fi	(552)	AKCRNVAVLGQEMCTMAAHIAELQYQIKLEELLQEIADQADRLVGSAAADLSS-FTEMLTQMDMRTTRL
PtIP-50Fj	(585)	AKCRNVAVLGQEMCTTAAHIGELTYQIKVVEMLQEIQAQADRLVGSAAADLSS-FTEMLSEMDMRTTRL

Fig. 10l

	771	840
PtIP-50Aa	(577)	LLELIKRVLYIQNAALQYEXLQTP-APLNANPVTMOTVWGLVQOETAANGLLQMGAPS----DYTQEYA
PtIP-50Bd	(576)	LLELIKRVLYIQNAALQYEXLQTP-ARLNANPVTMOTVWTELVQOETTAITGLLQLGAPS----DYTQGYV
PtIP-50Bb	(576)	LLELIKRVLYIQNAALQYEXLQTP-APLNANPVTMOTVWGLVQOETTAITGLLQLGAPS----DYTQEYV
PtIP-50Ba	(577)	LLELIQVNLNIQNGAARVEYLQPA-APLNANPVTMOTVWGLVQOEAANGLLQLGAPS----DYTREYV
PtIP-50Bc	(576)	LVELIKVNLNIQNGAARVEYLQPA-APLNANPVTMOTVWGLVQOETAANGLLQLGAPS----DYTREYV
PtIP-50Fb	(622)	LVELIKVLMQSVVALMQLSLTLP-ELMNAWPVTMETVWRMLIQHEHAANLGLMRLGPSF----DPRKLT
PtIP-50Fs	(629)	LVELIKVLMNQVVALMQLSLTMP-EDMNGWPVTMETVWRMLIQHEHAANLGLMRLGPSF----DPRKLT
PtIP-50Fo	(672)	QVELIKVLDVQVVALMQLSLVKP-ELMNAWPVTMDMVWRMLIQHEHAANLGLMRLGPSF----DPRKLT
PtIP-50Fk	(668)	LVELIKVLDMQVVALMQLSLIMP-EPINAWPVTMETVWGLVQHEHAANLGLMRLGPSF----DPRRTFT
PtIP-50Fe	(620)	LMELIKVLDMQVVALMQLSLTTP-TEMNANPVTMETVWGLVQHEHAANLGLMRLGPAF----DPRRTFI
PtIP-50Fl	(589)	LMELIKVLDMQNAALMQLSLTLP-APMNAWPVTMETVWGLVQHEHAANLGLMRLGPAF----DPRRTFI
PtIP-50Fn	(623)	LMELIKVLDMQNAALMQLSLSP-APMNAWPVTMETVWGLVQHEHAANLGLMRLGPAF----DPRRTFV
PtIP-50Ft	(654)	LVALINVVHMQNAALMQLCLSP-YYVNAWPVTMETVWSMLVQHEHAANLGLMRLGPSF----DPMRTYV
PtIP-50Fd	(663)	LVALIKVNIQNAALMQLSLSEP-TEVYANPVMDSVWRMLVQHEQFAIQGLMRLGPAF----DIVRTYV
PtIP-50Fm	(669)	LVALIKVNIQNAALMQLSLSEP-TEVYANPVMDSVWRMLVQHEQFAIQGLMRLGPAF----DIVRTYE
PtIP-50Fr	(665)	LVALIKVNIQNAALMQLSLSEP-TEVYANPVMDSVWRMLVQHEQFAVQGLMRLGPAF----DIVRAYV
PtIP-50Fg	(674)	LVALIKVMIQNAALMQLSLSEP-TEVSAWPVMMDTVWRMLVQHEQLAIQGLLRLGPAF----DIVRTFV
PtIP-50Fh	(676)	LVALIRAVHIQNAALMQLSLSEP-TEVSAWPVMMDAVWRMLVQHEQSAIQGLLRLGPAF----DIERVFV
PtIP-50Fp	(613)	LENLKVLTLQSGALQHEHLLP---TPFTGWVNMGMVRNALVKEHEADAVAAQERLGPST----DIVRTYV
PtIP-50Ga	(652)	LESLENVLAGHQGALHSHYIMELEVFTFSWPSVDSVRMHELQLNQRARARESGLFNGVLT-VLEENYV
PtIP-50Gb	(652)	LESLENVLAGHQGALHSHYIMELEVFTSSWPTVDSVRTHELQLNQRARARENALFEDGVLT-VLEQNDYV
PtIP-50Gc	(652)	LESLENVLAGHQGALHSHYIMELEVFTSSWPSVDGVRMHELQLNQRARARENALFEDGVLT-VLEQNDYV
PtIP-50Gd	(654)	LESLENVLADHQGALHSHYIMELQVFTFTWPSVDSVRMHELRLNQWARARENMLEFEDGVLT-VLEVQDDYV
PtIP-50Fa	(637)	LFQLIKVLIHQNAALKEEYLYAADEHLVSWPVSMTVWTMLLQQORLSLGLWENLVGYNLNPNDTRTYV
PtIP-50Ff	(644)	LLELIKMLYIQNAALIKYEYLYDANEKLSWPSMETVWTMLLQENAAALGLLDLGPIN----DFTVTYA
PtIP-50Fq	(641)	LLELIKMLYIQNAALIKYEYLYDANEKLSWPSMETVWTMLLQENAAALGLLNLGPSN----DFTVTYA
PtIP-50Fi	(621)	LLQLIKLLHIQNAALIKYEYLYPANGRLSSWPTMRTVWEMLQOESSAITELLALGPST----DPRRTFV
PtIP-50Fj	(654)	LLQLIRLLHIQNAALIKYEYLYAAGEQLNSWPSMDTVWSMLLQENSAALVGLLDLGPSN----DFTSFFV

Fig. 10m

	841	910
PtIP-50Aa	(642) VRDVPVRELLGGGDWEFELPVRNAD--FPLTWCVRIRIVVDWFDAAAE-----HLPVSTGGEV	
PtIP-50Bd	(641) VENIPVSELLLEGRDWEFELPVRNAD--FPTTWCVRIRIHEVDMQFDAGAK-----HLPSSTGQGV	
PtIP-50Bb	(641) VKDIPVSELLLEGRDWEFELPVLNAD--FPTTWSRVRIHEVDMQFDAAATSI-----HIPTENTGVV	
PtIP-50Ba	(642) VGDIPVKLLGGGDWEFELPVTAD--FPLTWCVRIRIQVDMQFDAAAE-----HLPTSTGGEV	
PtIP-50Bc	(641) VRDIPVRELLGGGDWEFELPVTDD--FPLTWCVRIRIHYVDMQFDAAAE-----HLPTSTGGEV	
PtIP-50Fb	(687) VKDIPVDLLHGEDWEFEISVDDFT-VFPRTWSRVRIHELEMKFVGS-DEAAPGGMQGANQPAKTEGEV	
PtIP-50Fs	(694) VKDIPVDLLHGEDWEFEISVDDFT-AFPATWSRVRIHELEMKFVGS-EEAPPWG-MQGP HQPAKTSGEI	
PtIP-50Fo	(737) VKDIPVDLLHGEDWEFEIPVDDFR-VFPATWSRVRIHELEMKFAAA-----PAG-TQGANQPAKTSGEI	
PtIP-50Fk	(733) VKDIPVDLLHGEDWEFEIPVDDFT-VFPV TWSRVRIHELEMKFVGT-DEAAPAPGLQGT HQPTKTEGEI	
PtIP-50Fe	(685) VKELPVDLLHGEDWQFEIPVNER T-VFPSTWSQVRIHELEMKFVGSNN-----IDHEGSHQPTTESGEV	
PtIP-50Fl	(654) VKELPVDLLHGEDWQFEIPVNE LT-VFPGTWSQVRIHELEMKFVGTAE G-----SAPNGANLPTTESGEV	
PtIP-50Fn	(688) VKELPVDLLHGEDWQFEIPVNE LT-VFPGTWSQVRIHELEMKFVGTAE G-----SAPNGANQPTTESGEV	
PtIP-50Ft	(719) VEGIPVQLLDGEDWEFEISVEDYT-TFPTTWSRVRIHELEMKFVQAAD-----VHQPTDITGKV	
PtIP-50Fd	(728) VKSIPVSELLLDGDDYEFEPVENHI-TFPLSLSRVRIHELEMKFVQGD TA-QG---AHVHMPTDITGSI	
PtIP-50Fm	(734) VKGIPVSELLLDGDDYEFEPVPAQDHV-TFPLSLSRVRIHELEMKFVQGE G-----DQLHMPVDTGSV	
PtIP-50Fr	(730) VKSIPVSELLLEGDDYEFELPVEDHI-TFPLSLSRVRIHELEMKFVQGAAGG-SDQGTQIVHMPTDITGSI	
PtIP-50Fg	(739) VKSIPVRELLLDGDDYQFEIPVEDPL-TFPLSLSRVRIHELEMKIVQGAH-----VHTPTATGKI	
PtIP-50Fh	(741) VEDI PVSELLLEGDDYEFEDIPVEDNI-TFPLSLSRVRIHELEMKFVASSGQ-----KQVHMPVDTGSV	
PtIP-50Fp	(677) MSGIPVSELLLDGDDWIFETINPLDGS-TFPLSWSRVIRIHELEMKFTGEE-----FHKPTDITGKV	
PtIP-50Ga	(721) LDAIPVSELLSGEDWNETINPERNASSFPTPDYVRIRIVVEMFTGE-----HQPVKTEGEI	
PtIP-50Gb	(721) LDAIPVSELLSGEDWNETINPERNASSFPTPDYVRIRIVVEMFTGE-----HLPVSETGEI	
PtIP-50Gc	(721) LDAIPVSELLSGEDWSETINPERNASAFPTPDYVRIRIVVEMFTGE-----HLPVDTGGEI	
PtIP-50Gd	(723) VDAIPVSELLSGEDWSETINPQNASVFPA PDYVRIRIVVEMFTGE-----HHFVSGTGEV	
PtIP-50Fa	(707) VKDIPVENLLSGRDWPVVIHEEDYT-AFPGHWYVRIRIVVEMFTGEQQTAGS----TDIVINQPSNTIGRL	
PtIP-50Ff	(710) VKDIPVKLLVDGFDWNEFIAVEDFA-IFPGLSRVIRIVVEMFTDQQGADS----SNIVINQPSNTIGLV	
PtIP-50Fq	(707) VKDIPVKLLVDGFDWNEFIAVEDFS-IFPGLSRVIRIVVEMFTDQQGTDS----SNIVINQPSNTIGLV	
PtIP-50Fi	(687) VEDI PVGELVDGFDWQFAIPVMDSSAFFIGSRVIRIVVEMFTSAIDIDA----S-ATVHQPRDNGFI	
PtIP-50Fj	(720) IQGIPIGELVDGENDWQFSPVEDSP-TFPIGSRVIRIVVEMFTDQGTSDS----G-RMIHQPSNTIGLT	

Fig. 10n

	911	980
PtIP-50Aa	(699) YMLLQSSRFFEDRAKRENEFISYEGGMGLQYQYAYRLATGDAIVTINVPSSEYANTFMRLAPFF-RWRRLRL	
PtIP-50Bd	(698) YMLLQSSRFFADRAKRANEYINQAGTGLYQYAYRLATGDAIVTINVPSSEYANTFMRLAPFF-RWRRLRL	
PtIP-50Bb	(700) YMLLQSSRFFDDRARRANEFISYEAGTGLFYQYAYRLATGDAIVTINIPDEYANTFMRLTTPFF-RWRRLRL	
PtIP-50Ba	(699) YMLLQSSRFFEDRAQHEDEFISYEAGTGLQYQYAYRLATGDAIVTINVPSSEAYVNTFMLLAPFF-RWRRLRL	
PtIP-50Bc	(698) YMLLQSSRFFDDRQKHEDEFISYEAGTGLQYQYAYRLDPCDAIVTINVPSDAYVNTFMLLAPFF-RWRRLRL	
PtIP-50Fb	(756) YILLQSSRVFHDRNKT--KPLHNEAGIPLDYHYAYNLEPGETTLSNLP SHDFIRAFMRMTTPFF-TWRRLRV	
PtIP-50Fs	(761) YILLQSSRVFHDRNKT--KPLHNEAGIPLDYHYAYNLEPGETTLSNLP SYDFVRAFMRMTTPFF-AWRRLRV	
PtIP-50Fo	(800) YILLQSSRVFHDRNKA--EPLHNEAGVPLDYHYAYNLEPGETTLSNLP SYDFIRAFMRMTTPFF-TWRRLRV	
PtIP-50Fk	(801) YILLQSSRVFHDRNNT--KPLHNEAGVPLDYHYAYNLEPGETTLSNLP SYDFIRAFMRMTTPFF-TWRRLRV	
PtIP-50Fe	(749) YILLQSSRVFHDRNKS--ELHNEAAVPLDYHYAYHLEPGETTLSNLP SNEFIRTFMRMTTPFF-TWRRLRV	
PtIP-50Fl	(719) YILLQSSRVFHDRNKT--EPLHNEAAVPLDYHYAYHLKPGETTLSNLP SNEFIRTFMRMTTPFF-TWRRLRV	
PtIP-50Fn	(753) YILLQSSRVFHDRNKT--EPLHNEAAVPLDYHYAYHLKPGETTLSNLP SNEFIRTFMRMTTPFF-TWRRLRV	
PtIP-50Ft	(778) YMLLQSSRIFRDRNRA--DAIRNEAAMSLDYHYAYRLDPCETTLSNLP SQDFTRTFMRMTTPFF-NWRRLRL	
PtIP-50Fd	(793) YILLQSSRNFRDRNEG--ALHNEAATPLDYHEAYRLDPCETTIVTNLP SAEFLTFMRMTTPFF-NWGLRLV	
PtIP-50Fm	(795) YILLQSSRNFRDRSEG--TILHNEAATSLDYHEAYRLDPCETTIVTNLP SAEFLRTFMRMTTPFF-NWRRLRV	
PtIP-50Fr	(798) YILLQSSRNFRDRNES--ALHNEAATSLDYHEAYRLDPCASIVTNLP SAEFLTFMRMTTPFF-NWRVRV	
PtIP-50Fg	(798) YILLQSSRNFRDRNEG--KIMHNEAATALDYQYAYQLDPCETTIVTNLP SAEFLRTFMRTTPFF-NWRRLRL	
PtIP-50Fh	(803) YILLQSSRNFRDRNER--AVVHNEAATSLDYHYAYNLEPGQIKVTNQ SAEFRKTFMRSTTPFF-NWRRLRL	
PtIP-50Fp	(736) YMLLQASRNFEQDRLEP--EVLHNEAAVPLQYQYAYHLEPGETTIVENRPSQQHGEYMQMTTPFG-QWRRLRL	
PtIP-50Ga	(778) YLVLRSSANEQDRLEE--QVLENEAAVPLVYQYAYNLSGCATLPLNLPSES--GKFFEMTTPFF-RWRRLRL	
PtIP-50Gb	(778) YLLLRSSANEQDRLEE--QVLENEAAVPLVYQYAYNLSGCATLPLNLPSES--GKFFEMTTPFF-RWRRLRL	
PtIP-50Gc	(778) YLLLRSSANEQDRLEE--QVLENEAAVPLVYQYAYNLSGCATLPLNLPSES--GKFFEMTTPFF-RWRRLRL	
PtIP-50Gd	(780) YLLLQSSANEQDRFEG--QVLENEAAVPLVYQYAYNLTGCATLPLNLPFES--GKFFEMTTPFF-RWRRLRL	
PtIP-50Fa	(772) YMLLQSSRFFHDRNQG--VKMEVEGSRGLSPYAYNLTGCTTHNNIPSDQFKNIEMQMTTPFF-TWRRLRL	
PtIP-50Ff	(775) YMLLQSSRFVHDRKQ--EVMDEASTGPVYAYAYDLNFGATLNNIPSQQANTFMQMTTPFN-AWRRLRL	
PtIP-50Fq	(772) YMLLQSSRFLHDRKRE--EVMDEASTGAVYAYAYDLNFGATLNNIPSQQANTFMQMTTPFNAAWRRLRL	
PtIP-50Fi	(752) YILLQSARSFSDRVR--QAMDYEASEGLAPYAYNLTGCVESLTNLP SQEANTFMEMTTPFF-EWRRLRL	
PtIP-50Fj	(784) YMLLQSSPVFRDRRR--EVLNEASMGLEYAYAYNLTGCVESLTNLP SFEANTFMEMTTPFN SNWRRLRL	

Fig. 10o

	981	1038
PtIP-50Aa	(768) SASAPENKGLAFP-TAFLAD---ATTRKITEFHVSAIRRLSTRVAV-----	
PtIP-50Bd	(767) SASADENKGLAFP-TATSAD---ATTQIKITEFHVSAIRRLSTFVADPK-----	
PtIP-50Bb	(769) SLSAENAGLAFP-TATSAD---DTTQIKITEFHVSAIRRLSTRSDGVSS-----	
PtIP-50Ba	(768) SSSAPENKGLAFP-TATSAD---ATTRKITEFHVSAIRRLSLAR-----	
PtIP-50Bc	(767) STSAPENKGLAFP-TATSDD---ATTRKITEFHVSAIRRLSLSR-----	
PtIP-50Fb	(823) SASAQENEGLAFPPTATVCGAG---DTTQIAITTFHVSAIREIAL-----	
PtIP-50Fs	(828) SASAQENEGLAFPPTATVCGAG---HTTQIAITTFHVSAIREIAF-----	
PtIP-50Fo	(867) SASAQENEGLAFPPTATVGTG---DTTQIAITTFHVSAIREIAL-----	
PtIP-50Fk	(868) SASAQENEGLAFPPTATAGNG---DTTQIAVTFHVSAIREIAL-----	
PtIP-50Fe	(816) SASAQENQGLAFPPTTFVCGAG---DTTQIAVTFVSAIREISL-----	
PtIP-50Fl	(786) SASAQENQGLAFPPTTFVCGAG---DTTQIAVTFVSAIREISL-----	
PtIP-50Fn	(820) SASAQENQGLAFPPTTFVCGAG---DTTQIAVTLVSAIREISL-----	
PtIP-50Ft	(845) SASAENKGLGFPTATS-AG---STTQIAITTFYSAIRRLSL-----	
PtIP-50Fd	(860) SASAENKGLAFSTATSAD---ATTQIAITTFISAIRQIAL-----	
PtIP-50Fm	(862) SASAENKGLAFPPTATSAD---ATTQIAITTFISAIRQIAL-----	
PtIP-50Fr	(865) SASAENKGLAFPPTATSADA---ATTQIAITTFISAIRQIAL-----	
PtIP-50Fg	(865) SASADENEGLAFPPTATSAD---ATTQIAITTFHISAIRRLSD-----	
PtIP-50Fh	(870) SASAENKGLAFPPTATSAD---ATTQIVVTFHISAVRQVAF-----	
PtIP-50Fp	(803) SASAFENRNLAFPPTAANTDLD-AATTQISITTFVTAVRANDFRSLDDQDM-----	
PtIP-50Ga	(843) SASAYQNEGVSFPTLPGLPGAADSPVQITITTFYVTALPQIQTS-STDQDPASTT----	
PtIP-50Gb	(843) SASAYQNEGVSFPTLPGLPN-SDSPVQITITTFYVTALPQIQSR-SLMSDGDPTEE--	
PtIP-50Gc	(843) SASAYQNEGVSFPTIPGDPPTSADTFVREITITTFYVTALPQIQSRSLDMST-----	
PtIP-50Gd	(845) SASAYQNEGVSFPTANSSDT--S--IQITITTFYVTALPQIQSL-SEDPVAVTTL----	
PtIP-50Fa	(839) SASAENEGLVFHPSTSPPT---STTQVSITTFHVTRIRREIRFTSTVHQKHAS-----	
PtIP-50Ff	(842) SASAENQGLVFP-TATSPD---NTTQISITTFYVTAIRREIRLRQEGDVE-----	
PtIP-50Fq	(840) SASAMENQGLVFP-TATSPD---NTTQISITTFYVTAIRREIRHRQEGDEE-----	
PtIP-50Fi	(819) SSSAENRGLTFP-TATSPD---DSTQISITFHITAIRREIRFRGMDDEERSKSDSSQK	
PtIP-50Fj	(852) SSSAMENQGLMFP-TASSAD---DTTQITITTFISAIRREIRTRAAVEI-----	

Fig. 11a

	1		50
PtIP-50Aa	(1)	MAAGDYSVLYQDVNQISIRLEKMDFSEVMAVHRMFVRMDDLVDVSSGTGLL	
PtIP-50Ba	(1)	MADLDYSKLYQDINQISVRLEKTEFSEVMVHRMFVRMDDLVDVSSGSGLL	
PtIP-50Bc	(1)	MADV DYSKLYHDINQISIRLGNVEFSEVMVHRMFVRDDLDVSSGSGLL	
PtIP-50Bb	(1)	MADIDYSVLYNDVNQISIRLERMDFSEVMAVHRMFVRMDDLVDVSSGTGVL	
PtIP-50Bd	(1)	MAGIDYSVLYQDVNQISIRLEKMDFSEVMAVHRMFVRMDDLVDVSSGTGLL	
	51		100
PtIP-50Aa	(51)	SGAEKVKRLYVFADVVELPSKQRLRLEGTDELIVILCRIFVTNGRHSTELFL	
PtIP-50Ba	(51)	SGAEKVKRLYVFADVVELPSKQVRLAGTDMIIVFCRIFVPEGRHYAELFL	
PtIP-50Bc	(51)	SGAEKVKRLYVFADVVELPSKQVRLAGTDMIIVFCRIFVINGHHGTELFL	
PtIP-50Bb	(51)	EGAQNVKRLYVFADVVELPSKQVRLPGSDMIVILCRIFVRNGRHNTLFL	
PtIP-50Bd	(51)	AGA EKVKRLYVFADVVELPSKRVRLPGTDMIIILCRIFVVNGRHSTELFL	
	101		150
PtIP-50Aa	(101)	PSMNMSMVAPG-TGSIRGVILSPTTVLTTSSDALQFKLQSGSMTSVMRLK	
PtIP-50Ba	(101)	PSMNMSMVGADGEGSIRGVILS-PTVLTTL SNALQFRLECGSMTSVMRLN	
PtIP-50Bc	(101)	PSTSMSMVAAG-EGSIRGVILS-PTTLTPLSHALQFKLESGSMTSVMRLN	
PtIP-50Bb	(101)	PSMNMSMVAAG-NGEIRGVNLS-TTMSSSSNALQFNLRSGSMTSTVRLK	
PtIP-50Bd	(101)	PSMNLNMVAAG-TGSIRGAYLS-TTAFSLSSNALQFKLQSGSMTSAIQLK	

Fig. 11b

		151		200
PtIP-50Aa	(150)	DVSVAATLTCNVQAASASMP	LVKTTGTSPGNICVLGMSTAVV	PESAVA
PtIP-50Ba	(150)	DVSAGATLTCNVQAASACV	PLKVKTTGTSPGNICVLGLSTA	AVVPE
PtIP-50Bc	(149)	DVSVAATLACNVQAASASMP	LKVKTTGTSPGNICVLGMSTDA	VVPENVKA
PtIP-50Bb	(149)	DVDVAAALTCDVQAASASMP	LVMTTGTSPGNIWVLMGTTAVV	IPESAVA
PtIP-50Bd	(149)	DVDLAATLTCDVQAASASMP	IVVKTGTSPGNILVLMSTAVV	IPESAVA
		201		250
PtIP-50Aa	(200)	VITDANILLGMQVTVLIAEL	VKIAHNSDVLIAAVTRHVEWLN	HLLVQAHA
PtIP-50Ba	(200)	VITDANILLGMQVTVLIAEL	VKIAHNSDGVIAAVTRHVEWLN	HLLVQAOA
PtIP-50Bc	(199)	VMVDVNILMGKQVTVLIAEL	VKIANNSDAVIAAVTRHVEWLN	YLLVQALV
PtIP-50Bb	(199)	VITDANILLGMQVTVLIAEL	VKTAHNSDVIIAAVTRHVEWLN	HLLVQAHA
PtIP-50Bd	(199)	VITDANILLGMQVTVLIAEL	VKTAHNSDVVIAAVTRHVEWLN	HLLVQAHA
		251		300
PtIP-50Aa	(250)	AAPSEDVVALLYRTQGF	IKLRNEGLIVPRLQYRMYKDL	IDRMVQVAQSYD
PtIP-50Ba	(250)	AAPSEDVVALLYRTQAF	IKLRKEGLIVPRLQYHMYKDL	IDRMVQVAQSYD
PtIP-50Bc	(249)	AAPSDDVVMLLYRTQAL	IKLQKEGLIVPRLQYHKYKDL	IDRMVQVAQSYD
PtIP-50Bb	(249)	AAPNEDVITLLYRTQAF	IKLKREGLVVPRLQYHMYKNL	IDRMVQVAQNYD
PtIP-50Bd	(249)	AAPSDDVVALLYRTQGF	IKLQREDLVVPGVLPRLQYRMYKDL	INRMVQVAQSYD

Fig. 11c

		301		350
PtIP-50Aa	(300)	QDFKQLKLFVEQNKILGSYLLEQNKAFAEKEKMDAFHSQVIDLRTSELE		
PtIP-50Ba	(300)	QDFKQMKLYVEQNKILGSYLLEQNKAFAEKEKMDASHSQVIALRTSELO		
PtIP-50Bc	(299)	HDFKQLKLYVEQNKILGSYLLEQNKAFAEKEKMDASHSQVIALRTSELE		
PtIP-50Bb	(299)	QDFRQLKLFVEQNKILGSYLLEQNKAFAEKEKMDAFHSQVIALRTTELN		
PtIP-50Bd	(299)	QDFKQLKLYVEQNKILGSYLLEQNKAFAEKEKMDAFHSQVIALRTTELN		
		351		400
PtIP-50Aa	(350)	STIERMDDLKQMEEQNAAMEQAKADMDAGLIAYQNKQVANAVFAVLGAI		
PtIP-50Ba	(350)	STIERMDDLKQMEVQSTAMEKAKADMDAGLIVYQNKQVADAVFAVMEAI		
PtIP-50Bc	(349)	STIERMSDLKQMEVQSKAMKQAKADMDAGLVEYQDKQVADAVFAVLEAV		
PtIP-50Bb	(349)	NTIERMGELSKQMQENEAMEQAKADMDAGLIEYQNRQVANALFAVLGAI		
PtIP-50Bd	(349)	NTIERMDDLKQMEEQNAAMEQAKADMDAGLIEYQNKQVANAVFAVLGAI		
		401		450
PtIP-50Aa	(400)	ASIGLAFATGGATAPGAVASAGA AVTAAGKAAEGLKKVVEIIEGLQAVME		
PtIP-50Ba	(400)	ASIGLAFATGGATAPGAVASAGA AVSAAGKAGEGLKKVVEIIEGLQAVME		
PtIP-50Bc	(399)	ASIGLAVATGGASAPEAVASAGA AVSAAGKAAEGLKKVVEVIEGLKAVME		
PtIP-50Bb	(399)	ASIGLAFATGGATAPGAVSAAGA AVTAAGKAAEGLKKVVEIIEGLQAVME		
PtIP-50Bd	(399)	ASIGLAFATGGATAPGAVAAAGTAVTAAGKAAEGLKKVVEIIEGLQAVME		

Fig. 11d

		451		500
PtIP-50Aa	(450)	VVAVIKELVQSLQEI	QGLVDAPEMPDLPS	DAEWEIFVNEVEAVAEQMPTE
PtIP-50Ba	(450)	VIAAIKGLVQSLQK	IGQLVNAPEMPDL	PSEAWEFMFVKEVEAVAAQMPTE
PtIP-50Bc	(449)	VIAAINKLVQSIQK	IGQLVSAPEMPD	LPSDAKWEIFVNEVEAVAAQMPKE
PtIP-50Bb	(449)	VVAAIKELVQSLQ	QIGQLVDAPEMP	DLPSNAEWEIFVNEVEAVAEQMPTE
PtIP-50Bd	(449)	VVAAIKELVQSLQ	EIGQLVNAPEMP	DLPSNAEWDIFVNEVEAVAEQMPTE
		501		550
PtIP-50Aa	(500)	VTEVPAWKAKCKN	VAAALGREMSTMA	AHIAELQFEIQVQEMLREIAKKQAD
PtIP-50Ba	(500)	VTQVPAWTAKCKN	VAAALGREMSTTA	AHIAELQYEIQVQGMLOQI
PtIP-50Bc	(499)	VSQVPAWTAKCKN	VAAALGREMSTTAV	HISELQYEIQVQGMLOEIAKKQAE
PtIP-50Bb	(499)	VTQVAAWKAKCKN	VAAALGREMSTMA	AHIAELQYQIQVQEMLREIAQKQAD
PtIP-50Bd	(499)	VTEVPAWKAKCKN	VAAALGREMSTMA	AHISELQFEIKVQEMLREIAQKQAD
		551		600
PtIP-50Aa	(550)	RLSSIKPADLTNY	LEMVSEMDMRTR	MLLELIRVLYIQNAALQYEYLQTP
PtIP-50Ba	(550)	RLSSIKPADLTNY	FEMVSEMDMRTR	MLLELIQVLNIQNGALRYEYLQPA
PtIP-50Bc	(549)	RLSSIKPADLTNY	LEMVSEMDMRTR	MLVELIRVLNIQNGALRYEYLQPA
PtIP-50Bb	(549)	RLSSISPADLTNY	LEMVSQMDMRTR	MLLELIRVLYIQNAALQYEYLQTP
PtIP-50Bd	(549)	RLSSIKPADLTNY	LEMVSEMDMRTR	MLLELIKVLYIQNAALQYEYLQTP

Fig. 11e

	601		650
PtIP-50Aa	(600)	APLNAWPVTMQTVWGLLVQOETAANGLLQMGAPSDYTQEYAVRDVPVRL	
PtIP-50Ba	(600)	APLNAWPVTMQTVWGLLVQOQEAANGLLQLGAPSDFTREYVVGDI PVKL	
PtIP-50Bc	(599)	APLNAWPVTMQTVWGLLVQOETAANGLLQLGAPSDYTREYVVRDIPVRL	
PtIP-50Bb	(599)	APLNAWPVAMQTVWGLLIQOETTAITGLLQLGAPSDFTQEYVVKDIPVSL	
PtIP-50Bd	(599)	ARLNAWPVTMQTVWTLVQOETTAITGLLQLGAPSDYTQGYVVENIPVSL	
	651		700
PtIP-50Aa	(650)	LLGGDWEFELPVRNADFPLTWCRVRIHYVDMRFDAAAE--HLPVTSTGE	
PtIP-50Ba	(650)	LLGGDWEFELPVTDAADFPLTWCRVRIQHVDMQFDAAAE--HLPPTSTGE	
PtIP-50Bc	(649)	LLGGDWEFELPVTDDDFPLTWCRVRIHYVDMQFDAAAE--HLPPTSTGE	
PtIP-50Bb	(649)	LLEGRDWEFELPVLNADF PSTWSRVRIHHVDMQFDDAAATSIHIPTNTGV	
PtIP-50Bd	(649)	LLEGRDWEFELPVMNADFPTTWCRVRIHHVEMQFDAGAK--HLPSTSTGQ	
	701		750
PtIP-50Aa	(698)	VYMLLQSSRFFEDRAKRENEFISYEAGMGLQYQYAYRLATGDATVTNVPS	
PtIP-50Ba	(698)	VYMLLQSSRFFEDRAQHEDEFISYEAGTGLQYQYAYRLATGEATVTNVPS	
PtIP-50Bc	(697)	VYMLLQSSRFFDDRAKHEDEFVSYEAGTGLQYQYAYRLDTGEATVTNVPS	
PtIP-50Bb	(699)	VYLLQSSRFFDDRARRANEFISYEAGTGLFYQYAYRLATGEATVTNIPT	
PtIP-50Bd	(697)	VYMLLQSSRFFADRAKRANEYINYQAGTGLYYQYAYRLATGEATVTNIPS	

Fig. 11f

		751		800
PtIP-50Aa	(748)	EEYANTFMRLAPFTRWRLRLSASAPENKGLAFPTATLADATTRIKITFHV		
PtIP-50Ba	(748)	EAYVNTFMLLAPFTRWRLRLSSAPENKGLAFPTATSADATTRIKITFHV		
PtIP-50Bc	(747)	DAYVNTFMLLAPFTRWRLRLSTSAPENKDLAFPTATSDDATTHIKITFHV		
PtIP-50Bb	(749)	DEYANTFMRLTPFTRWRLRLSLSAEENAGLAFPTATSADTTQIKITFHV		
PtIP-50Bd	(747)	EEYANTFMRLAPFTRWRLRLSASADENKGLAFPTATSADATTQIKITFHV		
		801		815
PtIP-50Aa	(798)	SAIRRISTRVAV---		
PtIP-50Ba	(798)	SAIRRISLAR-----		
PtIP-50Bc	(797)	SAIRRISLSR-----		
PtIP-50Bb	(799)	SAIRRISTRSDGVSS		
PtIP-50Bd	(797)	SAIRRISTFVADPK-		

Fig. 12a

	1	50
PtIP-50Fb	(1) -----	MGG
PtIP-50Fs	(1) -----	MGG
PtIP-50Fe	(1) -----	MER
PtIP-50Fl	(1) -----	
PtIP-50Fn	(1) -----	MER
PtIP-50Ft	(1) -----	MAA
PtIP-50Fk	(1) EIETEKVLIVCERVAYLFLGGWGACSEAPYRAIGILSADQNPHEIFTMGG	
PtIP-50Fo	(1) --RERGVNLGMLTSDHPFWGDWVCSEA-HRSICLVCPDQSLHEIA-MGG	

	51	100
PtIP-50Fb	(4) AVPDYSHLYRELNQVSEGMKLDQMEFSEVMVIHRMFIRLHDLNIAHREGA	
PtIP-50Fs	(4) AVPDYSHMYRELNQVSERMRLDHIEFSEVMVIHRMFIRLPDLIDIALQEGA	
PtIP-50Fe	(4) AVPDYSHMYRELNQVSEIRIMDQMEFSEVMVIHRMFIRLPDLSIAHLDGA	
PtIP-50Fl	(1) -----MVIHRMFFKLPDLIDIAHLDGA	
PtIP-50Fn	(4) AIPDYSHLYRELNQVSEIRIMDQMEFSEVMVIHRMFIKFPDLGIAHLEGA	
PtIP-50Ft	(4) AAREYSELYKDLNQISERIRLDQMEFSEVMVIHRMFIRLEELDIGHLEGA	
PtIP-50Fk	(51) AVPDYSHLYRELNQVSEIRIRLDQMEFSEVMVIHRMFIRLPDLNIAHLEGA	
PtIP-50Fo	(47) AVPDYSHMYRELNQVSERMRLDHMEFSEVMVIHRMFIRLPDLIDIALQEGA	

Fig. 12b

		101		150
PtIP-50Fb	(54)	EQVKRLYVFADVVELSGTS-----	LTLLPGTMMVVILCRVLSVRSF	
PtIP-50Fs	(54)	QQVKRLYVFADVVELPETSTSASA-EPVTLRLPGTMMVVILCRVLLFQGS		
PtIP-50Fe	(54)	DKVKRLYVFADVVELGSVQ-----	QTSSLPGSIMVVIMCRVLTLHVD	
PtIP-50Fl	(22)	DKVKCLYVFADVVELGSAG-----	QTSNLPGSIMVVIMCRVLTLHAD	
PtIP-50Fn	(54)	DKVKCLYVFADVVELGSVR-----	QTSSLPGSIMVVIMCRVLTLHLD	
PtIP-50Ft	(54)	ERVKRLYVFADVVEVEEGGN--FIETGPLARLPGSIMVVILCRVLSVRVL		
PtIP-50Fk	(101)	GOVKRLYIFADVVELSGHL-----	VTTQLPGTMMVVILCRVLSERSP	
PtIP-50Fo	(97)	QQVKRLYVFADVVELPEMSTSVTVSETVTLRLPGTVIVVILCRVLFYQG-		
		151		200
PtIP-50Fb	(96)	-----QSQHGLDFPHMRLHAV-PGDNQMLRIHRRNPDSAQYDDYPVVCVH		
PtIP-50Fs	(103)	-----YSAYDLDFPHMRLHAV-PGEAQILRIHRRNPDSAQYDDYPVVCVH		
PtIP-50Fe	(96)	PI---ATAYQCDFPHMRLHAF--FGSNGVRIDRRN-SDSVHDDYPVITVS		
PtIP-50Fl	(64)	WS---RTSYRFVFPHMRLHAI--FDLQQARIHRRN-SDSVHDDYPVITIS		
PtIP-50Fn	(96)	SA---AQAYRIDLPHMRLHAI--NDAQRLRIHRRN-SDSLHDDYPVITIS		
PtIP-50Ft	(102)	MFDNGNNAMRLQLPHMKLRVVEITTQPTTKLQIHR-ADAEDYQYPALCIH		
PtIP-50Fk	(143)	-----TARYGLNFPHMRLHAVFQSASRIHMERLI PDSAQYDAYPVVCVH		
PtIP-50Fo	(146)	-----FARYDLDFPHMRLHAVQPSGAGVLRMHLNPDQYDDYPVVCVL		

Fig. 12c

		201		250
PtIP-50Fb	(140)	ADVLEFPVTG-----	GRDIFHLGPLANFCMVTTFP----	PQSLEEA
PtIP-50Fs	(147)	ADVLEFPVTG-----	GRDIFHLGPLANFCMVVTHP----	QLSLVEA
PtIP-50Fe	(140)	ANVIEFSGTR-----	AETLFAIAPLRDPSMISS---	DLISNTVER
PtIP-50Fl	(108)	ANVIEFSGTRS-----	SSVLFGIGVFLDPSMVL-----	SDRHSIVER
PtIP-50Fn	(140)	ANVIEFSGTRS-----	LRDIFGIGPLQDPSMIITNT--	ETQPPVVER
PtIP-50Ft	(151)	ADVIQASIRLSSSSNRPPMDLRFFNFRPNRGS	DNVVFSS---	ESRSLIDE
PtIP-50Fk	(188)	ADALEFSGTS-----	TGGIFHIGPLRYPSMIVTSES----	HSAVDA
PtIP-50Fo	(191)	ADVVEFTRQR-----	PSRLFRIGPLAYPSMIFTDERPPASSIVEPR	
		251		300
PtIP-50Fb	(177)	PVSSFTGSRVRLWTAASSPH----	RIDPNNLTFTPGFMVQGGSFNLPFN	
PtIP-50Fs	(184)	PVSSFTGSRVRLWSAATSSPH----	SIDPNNLTFTPSFSVQGGSFNLSFA	
PtIP-50Fe	(178)	PASGFFCNVVPNWG-QSPPI----	TVDPLRIRFQTGATMVGGGSLT--	FP
PtIP-50Fl	(146)	PSSHTLCNVNPVE--QSSPI----	MVDPDTIRFQSI FNWVGSNIMSPQVA	
PtIP-50Fn	(180)	PSINSLCTVGLVQCSPPV----	TVDPDRISRHFVQSVGGGALG--	TL
PtIP-50Ft	(198)	PVEIFYVYSHIPRFIHALPIPEAATVDPASR	NDWYVSLRRVNSTLSSS	
PtIP-50Fk	(225)	QISSFSCNVSLRSASASSPL----	KINPDNVSEQSMFNLVTGG--	GFGFA
PtIP-50Fo	(232)	PVSSFYCTIEFRWSSNN-----	IGPNSVGIGCVFRLSGGS--	TFSTG

Fig. 12d

		301		350
PtIP-50Fb	(223)	TQSE-----	DFWLLRRNLPDALLKDSSILLCMQTSMLIAELVEF	SHPSSD
PtIP-50Fs	(230)	TRSE-----	DFWLLQRNLPDALLKDSSILLCMQTSMLIAELVEF	SHPSSD
PtIP-50Fe	(221)	LGPE-----	GFSILQRNLPDLLLLIDPNILLCMQTSMLVAELVEF	SHPSSD
PtIP-50Fl	(190)	RAPE-----	GFSIFRRDLPDLLLLIDPNILLCMQTSMLIAELVEF	SHPSSD
PtIP-50Fn	(224)	RAAE-----	GFSIFQRDLPDVLLIDPNILLCMQTSMLVAELVEF	SHPSSD
PtIP-50Ft	(248)	VAAEGELEPAFSILKPKIPVVVLTDPNILLAMQTSLLIAELVEVAHPSAE		
PtIP-50Fk	(269)	VRPE-----	EFWLLRRDLPDALLNDSSILLCMQTSMLVAELVEF	SHPSSD
PtIP-50Fo	(273)	SPRE-----	DFWLFRRDLPDALFKDSSILPCMQTSMLVAELVEF	CHPSPD
		351		400
PtIP-50Fb	(268)	VRAAVTLHAEWLNELLQASAKS-	QGTPHHDDYRALLFRAQYVVVKG-	IGR
PtIP-50Fs	(275)	VRAAVTLHAKWLNTLLQASAKS-	EGTPHHADYRALLFRAQYVVVKG-	VGK
PtIP-50Fe	(266)	IHAAVTDHVAVLNTHLLQASAKA-	QGTSSHDDYLALIFRAQYLLKG-	MGR
PtIP-50Fl	(235)	IHAAVTEHLIWLNTHLLQASTKA-	QGTSSYDDYLALIFRSQYLLKG-	MGR
PtIP-50Fn	(269)	IHSAVTEHVVWLNTHLLQASTKA-	QGTSSHDDYLALIFRAQYLLKG-	MGR
PtIP-50Ft	(298)	TADAVAKHVEWLNKELLQATKVEGILNSSHDEHLALIFRAQYLMKMRPGR		
PtIP-50Fk	(314)	LHAAILHAEWLNTELLQASAAS-	QGTSSHDDYLALIFRAQYLLKG-	VGR
PtIP-50Fo	(318)	VRAAIRSHAKWLNTLLQASVAS-	EGTPHHSYNALLFRAQYVIKG-	VGK

Fig. 12e

	401	450
PtIP-50Fb	(316)	SRGAVVPQLQYDMYSNLINQARAADSYDQSLKQLKLFIAQNEILGEYLL
PtIP-50Fs	(323)	SRGAVVPQLQYDMYSNLVNQVARAADSYDQSLKQLQLFITQNEILGDYLL
PtIP-50Fe	(314)	ARSLVVPQLQYDVYRNLSQMARVAESYDQSLKQLQLFVAQNKILGGYLL
PtIP-50Fl	(283)	ARSLVVPQLQYDVYRNLINQMARVAESYDQSLKQLQLFLAQNKVLGGYLL
PtIP-50Fn	(317)	ARSLVVPQLQYDVYRNLINQMARVAESYDQSLKQLQLFLAQNKILGGYLL
PtIP-50Ft	(348)	TRNLVVPQLQYDVYSNLVNRMAQVAESYDQSLRQFKLFIDQNKILGSYLL
PtIP-50Fk	(362)	ARGAVVPQLQYDMYSNLINQARAADTYDQSLKQFQLFVAQNKILGGYLL
PtIP-50Fo	(366)	SRGAVVPQLQYDMYSNLVNQVARAADSYDQSLKQLQLFIAQNEILGEYLL

	451	500
PtIP-50Fb	(366)	EQNRVFAAKERDMEVFHSELIAQKTELEQTVVVKIDNLSLQMEAQVEDME
PtIP-50Fs	(373)	EQNRVFAAKERDMEVFHSELIAQKKTELETVMAKIDLLSLQMETQAADME
PtIP-50Fe	(364)	EQNRAFAAKERDMEVFHSELIAQKELELKNMVKMEQLSLQMETQIADMD
PtIP-50Fl	(333)	EQNRAFAAKERDMEVFHSELIAQKELELQNTMFKMEQLSSQMETQIADMD
PtIP-50Fn	(367)	EQNRAFAAKERDMEVFHSELIAQKELELQNTMFKMEQLSSQMETQIADMD
PtIP-50Ft	(398)	QQNKAFADKERDMEVFHSELISQKIELDNTYKMDQLSLQMESQREDMD
PtIP-50Fk	(412)	EQNRAFAAKERDMEVFHSELIAQKKTELEQTVVVKIDKLSLQMDTQVADME
PtIP-50Fo	(416)	EQNRVFAAKERDMEVFHSELISQKETELRTVMAKIDLLSLQMETQVADME

Fig. 12f

	501	550
PtIP-50Fb	(416)	QAREDMEAGLRREFNRQVANAMFSVFRAIGAVALTVLTGGAAAPLAISAA
PtIP-50Fs	(423)	QAREDMEAGLRREFNRQVANAMFAVFRAIGAVALTVLTGGAAAPLAISTA
PtIP-50Fe	(414)	QAKEDMDAGLRREFQNRQVANAMFAVFRAIGATALTVVTAGAAAPAAMAAA
PtIP-50Fl	(383)	QAEKDMEAGLRREFQNRQVARAIFAVFRAIGAVALTVVTGGAAAPAAMSAA
PtIP-50Fn	(417)	QAEKDMEAGLRREFQNRQVARAMFAVFRAIGAVALTVVTGGAAAPAAMTAA
PtIP-50Ft	(448)	QAKKMDAGLREFQNRQVANAMFAVIGATAAIGLAFITGGATAPAAVGAA
PtIP-50Fk	(462)	QAREDMEAGLRREFDRQVANALFSVFRAIGAVALTVLTGGAAAPLAISAA
PtIP-50Fo	(466)	QAREDMEAGLRREFNRQVANALFSVFRAIAAVALTVVTGGAAAPLAMSTA
	551	600
PtIP-50Fb	(466)	KGAVSIAGQAARGLERVLRILDDLQAAMELLKIIKDLVESLQEI GQLVDA
PtIP-50Fs	(473)	KGAVSIAGQAARGLQRVLQILDDLQAAMELLKIIKDLVESLQEVGQLVDA
PtIP-50Fe	(464)	KGAVTTAGQAARGLVRVLEILDDLQAAMEVFKIIKDLVESLREVGQLVDA
PtIP-50Fl	(433)	KGAVSIAGQAARGLERVLEILDNLQAAMEVFKIIKDLVESLREVGQLVDA
PtIP-50Fn	(467)	KGAVSIAGQAARGLERVLEILDNLQAAMEVFKIIKDLVESLREVGQLVDA
PtIP-50Ft	(498)	KTAVTAAGAAARALERVVEILDSLQAVMEIVSIIKELVSSLQEI GQLVEA
PtIP-50Fk	(512)	KGAVSIAGQAARGLERVLRQILDDLQAAMELLKIIKDLVESLQEVGQLVDA
PtIP-50Fo	(516)	KGAVSAAGQAARGLERVLRQILDDLQAAMELLKIIKDLLESQAVGQLVDA

Fig. 12g

	601	650
PtIP-50Fb	(516)	PDMPEMPTEADWAI FVNEIEGVAEQMPPEEVSEVSAWKT SCKNVAAVGREL
PtIP-50Fs	(523)	PDMPEMPTEADWAI FVNEIEGVAEQMPPEEVSEVSAWKT SCKNVAAVGREL
PtIP-50Fe	(514)	PDMPDMPTTEADWSIFVNEIEGVAEQMPPEEVSEVSAWKT SCKNVAAVGREL
PtIP-50F1	(483)	PEMPDMPTTEADWSIFVNEVEAVAEQMPPEEVSEVSAWKT SCKNVAAVGREL
PtIP-50Fn	(517)	PEMPDMPTTEADWSIFVNEVEAVAEQMPPEEVSEVSAWKT SCKNVAAVGREL
PtIP-50Ft	(548)	PDMPPMPTTEADWFI FENEIEGVAEQMPTEVSEVSAWKTCKKNVAVIGREM
PtIP-50Fk	(562)	PEMPDMPTTEVDWAI FVNEVEGVAEQMPPEEVSEVSAWKT SCKNVAAVGREL
PtIP-50Fo	(566)	PDMPEMPTEADWAI FVNEIEGVAEQMPPEEVSEVSAWKT SCKNVAAVGREL

	651	700
PtIP-50Fb	(566)	MTTAYMSQLQYDIQVQAMLQGIASKQADRLSSIQAADLSSFTTEMVTEMD
PtIP-50Fs	(573)	MTTAYMSQLQYDIQVQAMLQDIASKQADRLSSIQAADLSSFTTEMVTQMD
PtIP-50Fe	(564)	MTTSAYISQLQYDEKVQALLQDIANRQADRLSSIQAADLTSYTEMVTQMD
PtIP-50F1	(533)	TTTSAYISQLQYDEKVQAMLQDIANKQADRLSSIQAADLSSYTEMVTQMD
PtIP-50Fn	(567)	TTTSAYISQLQYDEKVQAMLQDIASRQADRLSSIQAADLSSYTEMVTQMD
PtIP-50Ft	(598)	TTTATYISELQYDEKVQEMLQEIATSHANRLSSIQATDLSNYTEMVTQMD
PtIP-50Fk	(612)	TTTSAYMSQLQYDVQVQAMLQDIARKQADRLSSIQAVDLSSFTTEMVTQMD
PtIP-50Fo	(616)	MTTAYMSQLQYDIQVQAMLQDIASKQADRLSSTQAADLSSFTTEMVTQMD

Fig. 12h

	701	750
PtIP-50Fb	(616)	MRTTRLLVELIKVLEHMQSV ALMYQSLTLP ELMNAWPVTMETVWRMLIQHE
PtIP-50Fs	(623)	MRTTRLLVELIKVENMQN VALMYQSLTMPEPMNGWPVTMETVWRMLIQHE
PtIP-50Fe	(614)	MRTTRLLMELIKVLD MQNVALMYQFLT PPTPMNAWPVTMETVWGMLVQHE
PtIP-50F1	(583)	MRTTRLLMELIKVLD MQNAALMYQFLT PAPMNAWPVTMETVWGMLVQHE
PtIP-50Fn	(617)	MRTTRLLMELIKVLD MQNAALMYQFL SLPAPMNAWPVTMETVWGMLVQHE
PtIP-50Ft	(648)	MRTTRMLVALIN VHMQNAALMYQCL SPPTYVNAWPVTMETVWSMLVQHE
PtIP-50Fk	(662)	MRTTRLLVELIKVLD MQNVALMYQSL IMPEPINAWPVTMETVWGMLIQHE
PtIP-50Fo	(666)	MRTIRLQVELIKVLD VQNAALMYQSL VVKPELMNAWPVTMDMVWRLLIQHE

	751	800
PtIP-50Fb	(666)	HAAVLGLIRLGP SDFRKT LTVKDI PVDLLLHGEDW EFEISVDDFTV FPR
PtIP-50Fs	(673)	HAAVLGLMRLGP SDFRKT LTVKDI PVDLLLHGEDW EFEISVDDFTA FPA
PtIP-50Fe	(664)	HAAVLGLMRLGP AFDFKRT FIVKEI PVDLLLHGEDW QFEIPVNERTV FPS
PtIP-50F1	(633)	HAAVLGLMRLGP AFDFRRT FIVKEI PVDLLLHGEDW QFEIPVNELTV FPG
PtIP-50Fn	(667)	HAAVLGLMRLGP AFDFRRT FVVK EIPVDLLLHGEDW QFEIPVNELTV FPG
PtIP-50Ft	(698)	HAAVLGLMRLGP SDFMRTYV VEGIPVQ LLDGEDW EFEISVEDYTT FPS
PtIP-50Fk	(712)	HAAVLGLIRLGP SDFRRT FTVKDI PVDLLLHGEDW EFEIPVDDFTV FPV
PtIP-50Fo	(716)	HAAVLGLMRLGP SDFRKT LTVKDI PVDLLLHGEDW EFEIPVDDFRV FPA

Fig. 12i

	801	850
PtIP-50Fb	(716)	TWSRVRIHHLEMK FVGS GDEAAPGGCMQGAHQPATKTGEVYILLQSSRVF
PtIP-50Fs	(723)	TWSRVRIHHLEMK FVGS -EEAAPWG-MQGP HQPATKSGEYIYILLQSSRVF
PtIP-50Fe	(714)	TWSQVRIHHLEMK FVGS NN-----IDHEGSHQ PITESGEVYILLQSSRVF
PtIP-50Fl	(683)	TWSQVRIHHLEMK FVGT AEG-----SAPNGAHL PITESGEVYILLQSSRVF
PtIP-50Fn	(717)	TWSQVRIHHLEMK FVGT AEG-----SAPNGA HQPITESGEVYILLQSSRVF
PtIP-50Ft	(748)	TWSRVRIHHLEMK FVQAAD-----VHQPI TD TGKVYMLLQSSRVF
PtIP-50Fk	(762)	TWSRVRIHHLEMK FVGT -DEAAPAPGLQGHQPTTKTGEYIYILLQSSRVF
PtIP-50Fo	(766)	TWSRVRIHHLEMK FAAA-----PAG-TQGAHQ PATKSGEYIYILLQSSRVF
	851	900
PtIP-50Fb	(766)	HDRNKTKPLHYEAGIPLDYHYAYNLETGETTLSNLPSHDFIRAFMRMTPF
PtIP-50Fs	(771)	HDRNKTKPLHYEAGIPLDYHYAYNLGTGETTLSNLPSYDFVRAFMRMTPF
PtIP-50Fe	(759)	HDRNKSELLHYEAAVPLDYHYAYHLETGETTLSNPSNEFIRTFMRMTPF
PtIP-50Fl	(729)	HDRNKTEPLHYEAAVPLDYHYAYHLKTGETTLSNPSNDFIRTFMRMTPF
PtIP-50Fn	(763)	HDRNKTEPLHYEAAVPLDYHYAYHLKTGETTLSNPSNDFIRTFMRMTPF
PtIP-50Ft	(788)	RDRNRADAIRYEAAVSLDYHYAYRLDTGETTLSNLPSQDFTRTFMRMTPF
PtIP-50Fk	(811)	HDRNNTKPLHYEAGVPLDYHYAYNLETGETTLSNLPSYDFIRAFMRMTPF
PtIP-50Fo	(810)	HDRNKAEPLHYEAGVPLDYHYAYNLETGETTLSNLPSYDFIRAFMRMTPF

Fig. 12j

	901	946
PtIP-50Fb	(816)	ATWRLRVSAQAENEGLAFPTATVGAGDTTQIAITFHVSAIREIAL
PtIP-50Fs	(821)	TAWRLRVSAQAENEGLAFPTATVGAGHTTQIAITFHVSAIREIAF
PtIP-50Fe	(809)	TTWRLRVSAQAENQGLAFPTTTVGAGDTTQIAVTFVSAIREISL
PtIP-50Fl	(779)	TTWRLRVSAQAENQGLAFPTTTVGAGDTTQIAVTFVSAIREISL
PtIP-50Fn	(813)	TTWRLRVSAQAENQGLAFPTTTVGAGDTTQIAVTLVSAIREISL
PtIP-50Ft	(838)	TNWRLRISASAEENKGLGFPTATS-AGSTTQIAIIFYISAIRRISL
PtIP-50Fk	(861)	TTWRLRVSAQAENEGLAFPTATAGNGDTTQIAVTFHVSAIREIAL
PtIP-50Fo	(860)	TTWRLRVSAQAENEGLAFPTATVGTGDTTQIAITFHVSAIREIAL

Fig. 13a

	1		50
PtIP-50Fd	(1)	-----MAAARSAGANEAWREYVYHDLNQVSDRIRLDQLEFSEVMVVRMHI	
PtIP-50Fg	(1)	-----MANDTSSQIVDWRELYRDMNQASDRIRFNQLEFSEVMVIYRMHI	
PtIP-50Fh	(1)	-----MASITSGAGADWRELYSEMNOVSEERERLDQLEFSEVMITHRMHI	
PtIP-50Fm	(1)	MQGRLASMAGSAGAADAWREYVRDLNQVSDRIRLDQLEFSEVMVIHRMHI	
PtIP-50Fr	(1)	-----MAARSAGADGWREYVRGLNQVSDRIRFDQLEFSEVMVIHRMHI	
	51		100
PtIP-50Fd	(46)	KMSELDLGHLEGAEKVERLYVFADMVECDVPPHASSSDGLVIRLPGSLS	
PtIP-50Fg	(45)	KMSELDMGHLEGSEKVKRLYVFADVVEYDAN----G-----IARLPGSIS	
PtIP-50Fh	(45)	KLSELDLGHLEGADKVHLYVFADVVELDNAADPSTP---VDVRLPGSIS	
PtIP-50Fm	(51)	KMSELDLGHLEGAEKVERLYVFADVVELDVVPHSSSDGDVVVRLPGSKS	
PtIP-50Fr	(45)	KMSELDLGHLAGAEKVERLEVFDVVECDVPPHENS\$SDPLVIRLPGSVS	
	101		150
PtIP-50Fd	(96)	VIFICRVWHINVGVVQLTS-----TVQMSRTLIVQLPHMRWHVFNATG\$	
PtIP-50Fg	(86)	VVFLCREWHVSYHLVARMDRNPPRYQLDLAQGVITSLPHMAWHLDVRRQ\$	
PtIP-50Fh	(92)	VVFLCRVWHVSRRLTVGVNSPQYWLPTFDGRPVRIQLPHVKWHMNTLRS	
PtIP-50Fm	(101)	VTFVCRVWHINYALVQISS-----FARISRSVLELRHMRWHFNAITGR	
PtIP-50Fr	(95)	VTFMCRVWHVNYGVVQRTS-----TVRVARTVVVQLPHMRLHFNATG\$	
	151		200
PtIP-50Fd	(140)	MHRLHPQTSQEGPDT\$FCVYAEVVQVAYY-----PDVTESEFDGSP	
PtIP-50Fg	(136)	MHRLDPETLQEFLEP\$FCVYADDISVAYYSLNGAS-WDPALQFPDTAELA	
PtIP-50Fh	(142)	MHRIDPETLQDL\$GPLL\$CVYADLLRTSLHPPVGH\$SNWDAALAFPSMTVLR	
PtIP-50Fm	(145)	MHLLDPQTLQDGM\$LCFCVYAEVVQIAYY-----PDVTEPESNDSP	
PtIP-50Fr	(139)	MHRLHPQTSQDGS\$TCFCVYAEVVQIAYY-----PDVTYPESNDSP	

Fig. 13b

		201		250
PtIP-50Fd	(181)	T-LRFLRLEFESDQLRVTTQT---	EGPTWATRASLDTTMTPDNDGLNEI	
PtIP-50Fg	(185)	KCALLIYSNSNPTPNHVLLTSNRFNGTRWAFRFEFS-MYYIDNEDVAKDI		
PtIP-50Fh	(192)	EVEVLPCFDHSTPRQGLLTLT---	SNAAPQWVNQLRAAFIFTDQDAMTEI	
PtIP-50Fm	(186)	SFLRFLRLEIVPDQLRVTTQT---	DGPVWALRGALEYFMAPDNDGLNEV	
PtIP-50Fr	(180)	VGLRFLRLEFGSDQLRVTRQS---	EGPVWALRGS LNYSMSPDNDGLMNEV	
		251		300
PtIP-50Fd	(227)	SAQPAGTIFKLNILPDNLIVSLFGSLRPNSSTSLTR-VPDNLPAFSIFR		
PtIP-50Fg	(234)	ALQPPGTRVIFKREGLHLVVLAMDSANRIISFDPERGDLDAYMPAFSLLR		
PtIP-50Fh	(239)	LSLPADASVILNKNRPSIILTAGQSG-----G--YT-DRNVSAPAFSVLR		
PtIP-50Fm	(233)	SSQPAGTSFVLNILEPDNLVQLFANLRPNSGTTLTR-DPNTIVPAFSVYK		
PtIP-50Fr	(227)	SSQPAGTSFILNILEPDNLNLQLMASLRNSNSTLTR-DPDNIVPAFSVYR		
		301		350
PtIP-50Fd	(276)	EKAV---ELLTDPDVL SAMQTSMLIGELVEVGQPPEATTEVVRKHIEWLN		
PtIP-50Fg	(284)	QDAKFPIELLTDPNLSAMQTSMLIGELVEVGNPSTAVTEVVHKKHVWLS		
PtIP-50Fh	(281)	EGAETPIDSLTDPNLF PAMQTSMLIAELVEVGRPSAAIIEVMHKKHVEWLN		
PtIP-50Fm	(282)	EKEG---ELLSDPNILEPAMQTSMLIGELVEVGQPPSVTTEVVRKHVEWLN		
PtIP-50Fr	(276)	EKLAV--ELLTDPNTLEPAMQTSMLIGELVEVGQPPEATIEVVRKHIEWLN		
		351		400
PtIP-50Fd	(323)	NLLLKVIEAKQG-QHVEDYVELSFRAQYVIKKNVGKIQRNVVPQLQYSAYS		
PtIP-50Fg	(334)	KLLHQVIEEKNG-KDVEDYIGLSFRAQYVIKKGVRIRGLVVPQLQYDVYS		
PtIP-50Fh	(331)	NLLQVIEAKKGDVEDYIQLSYRSQYILKNVGRIDRFVVPQLQYDTYS		
PtIP-50Fm	(329)	NLLQVIEAKRG-EHVEDYVELSFRAQYVIKKGVRIRQLVVPQLQYSAYS		
PtIP-50Fr	(324)	NLLLKVIEAKQG-EPVEDYIELSFRAQYVIKKGVRVQRLVVPQLQYSAYS		

Fig. 13c

	401		450
PtIP-50Fd	(372)	NLINRMAQVAESYDQALRQFKLFIQNN---KILGGFLEQNRAFAEKEQD	
PtIP-50Fg	(383)	NLINRMAQVAESYDQALRQFRLFIQNN---KILGGFLEQNRAFAEKEKD	
PtIP-50Fh	(381)	PLINRMAQVAESYDQALRQFRLFIQQTQQNKILGAFLLDQNRADFADKEKD	
PtIP-50Fm	(378)	NLINRLAQVAESYDQALRQFRLFIQNN---KILGGFLEQNRAFAEKEQD	
PtIP-50Fr	(373)	NLINRLAQVAENYDQALRQFRLFIQNN---KILGGFLEQNRAFAEREQD	
	451		500
PtIP-50Fd	(419)	MDVFYSELITQRQIELENTLQKMKHLGTQMDTQTADMEQAQADMEAG-VR	
PtIP-50Fg	(430)	MDVFYSELITQRQIELENTLQKLRSLQMETQQADMEQAQADMEAG-IR	
PtIP-50Fh	(431)	MDVFYSELIAQRQIELEHNTLQKMEQLSLOMETQADMEQAQADMEAG-IR	
PtIP-50Fm	(425)	MDVFYSELIAQRQIELENTLQKMKHLGAQMETQTADMEQAQADMEAG-IR	
PtIP-50Fr	(420)	MDVFYSELITQRKIELENTLQTMKHLGAQMETQTAGMEQAQADMEAGTR	
	501		550
PtIP-50Fd	(468)	KFQNEQVARGLFAVLGAI GAVGLTFLTGGAAAPLAISTARRAVSVAGAVA	
PtIP-50Fg	(479)	RFQNRQAARALFAVLGAI AAVGLAFLTGGATAPAAIGAARTAVTVAGSVV	
PtIP-50Fh	(480)	RFQNERVARAVFGVLGAI AAVALAFVTGGATAGAAIGAAKTAVTLAGAAA	
PtIP-50Fm	(474)	EFQNRQVARGLFAVLGAI AAVGLTLLTGGAAAPLAMSARSASVSLAGAVA	
PtIP-50Fr	(470)	RFQNAQVARGLFAVLGAI AAVGLTLLTGGAAAPLAVSAARRVVTVAGAVA	
	551		600
PtIP-50Fd	(518)	QGLQTVLDILEGLQAVMEIVHLINDLISALQELGQPVELPEMADMPTEAD	
PtIP-50Fg	(529)	SGLQRVLEVLEGLQAVMEIVVIKDLFSALQDLTQAVDLPDMPEMPLQSD	
PtIP-50Fh	(530)	RGLQQVLEILDGLQAVMEVVGMIKELFESLQELGQAVDLPMPEMPLQSD	
PtIP-50Fm	(524)	QGLQRVLDILEGLQVMEVVELINDLISSLQELGQPVELPEMAEMPTEAD	
PtIP-50Fr	(520)	QGLQRVLDILEGLQVMEVVELINDLISSLQDLGQPVLDPEMAELPTQAD	

Fig. 13d

	601		650
PtIP-50Fd	(568)	WLIFVNEVEGVAEQMPTEVSEVVAWKTCKKNVAVLGREMTTLAAYISQLQ	
PtIP-50Fg	(579)	WLIFVNEVEAVAQGMPTSEVVAWKTCKKNVAVLGQEMTTTAAAYISQLQ	
PtIP-50Fh	(580)	WHIFVNEVEAVAEQMPTEVSEVVAWKTCKKNVAVLGREMITLATYISQLQ	
PtIP-50Fm	(574)	WLIFVNEVEGVAEQMPTEVSEVVAWKTCKKNVAVLGREMTTLAAYISQLQ	
PtIP-50Fr	(570)	WLIFVNEVEGVAEQMPTEVSEVVAWKTCKKNVAVLGREMTTLAAYISQLQ	
	651		700
PtIP-50Fd	(618)	YDIEMQHMLQQIARKQADRLSAIQLPDLRN-YAELVTQMDMRTTRELVAL	
PtIP-50Fg	(629)	YDIMQDMLQQIARKQADRLSAIQLPDLTN-FTEMVIQMDMRTTRELVAL	
PtIP-50Fh	(630)	YDIQMDMLQQIARKQADRLSAIQLSIPNTGYTEMLMQMDMRTTRELVAL	
PtIP-50Fm	(624)	YDINMQHMLQQIARKQADRLSAIELPDLRN-YGELVTQMDMRTTRELVAL	
PtIP-50Fr	(620)	YDIQMDMLQQIARKQVDRLSAIQLPDLKN-YAELVTQMDMRTTRELVAL	
	701		750
PtIP-50Fd	(667)	IKVVNIQNAALMYQYLSEPTPVYAWPVNMDSVWRMLVQHEQFAIQGLMRL	
PtIP-50Fg	(678)	IKVMYIQNAALMYQYLSEPTPVSAWPVNMDTVWRMLVQHEQLAIQGLLRL	
PtIP-50Fh	(680)	IRAVHIQNAALMYQYLSEPIPVSAWPVNMDAVWRILVQHEQSAIQGLLRL	
PtIP-50Fm	(673)	IKVVNIQNAALMYQYLSEPTPVYAWPVNMDSVWRMLVQHEQFAIQGLMRL	
PtIP-50Fr	(669)	IKVVNIQNAALMYQYLSEPTPVYAWPVSMDSVWRMLVQHEQFAVQGLMRL	
	751		800
PtIP-50Fd	(717)	GPAFDIVRTYVVKSI PVSLLLDGDDYEFEPVENHITFPLSLSRVRIHHL	
PtIP-50Fg	(728)	GPAFDIVRTFVVKSI PVRLLLDGDDYQFEIPVEDPLTFPLSLSRVRIHHL	
PtIP-50Fh	(730)	GPAFDIERVFVVEDIPVSLLEGGDDYEFDIPVEDNITFPLSLSRVRIHHL	
PtIP-50Fm	(723)	GPAFDIVRTYEVKGI PVSLLLDGDDYEFEPVQDHYTFPLSLSRVRIHHL	
PtIP-50Fr	(719)	GPAFDIVRAYVVKSI PVSLLEGGDDYEFELPVEDHITFPLSLSRVRIHHL	

Fig. 13e

	801		850
PtIP-50Fd	(767)	EMRFVQDGDTAQG---AHVHMPITDTGSIYILLOGSRNFRDRNEGAILHY	
PtIP-50Fg	(778)	EMKIVQGAH-----VHTPITATGKIYILLOGSRNFHDRNEGKIMHY	
PtIP-50Fh	(780)	EMKFVASGGQ-----KQVHMPVTDSGSVYILLOGSRNFHDRNERAVVHY	
PtIP-50Fm	(773)	EMKFVQGEG-----DQLHMPVTDGTSVYILLOGSRNFHDRSEGTLHY	
PtIP-50Fr	(769)	EMKFVQGAAGGSDQGTQIVHMPITDTGSIYILLOGSRNFHDRNESAILHY	
	851		900
PtIP-50Fd	(814)	EAATPLDYHFAYRLDTGETTVTNLPSAEFLTTFMRMPFTNWGLRVSASA	
PtIP-50Fg	(819)	EAATALDYQYAYQLDTGETTVTNLPSAEFLRTFTRMPFTNWRLRLSASA	
PtIP-50Fh	(824)	EAATSLDYHYAYNLETGQIKVTNQPSAEFRKTFMRSTPFTNWRLRLSASA	
PtIP-50Fm	(816)	EAATSLDYHFAYRLDTGETTVTNLPSAEFLRTFMRMPFTNWRLRVSASA	
PtIP-50Fr	(819)	EAATSLDYHFAYRLDTGASTVTNLPSAEFLTTFMRMPFTNWRVRSASA	
	901		936
PtIP-50Fd	(864)	EENKGLAFSTATSAD-ATTQIAITFFISAIHQIAL-	
PtIP-50Fg	(869)	DENEGLAFPTATSAD-ATTEIAITFHISAIRRID-	
PtIP-50Fh	(874)	EENKGLAFPTATSAD-ATTQIVVTFHISAVRQVAF-	
PtIP-50Fm	(866)	EENKGLAFPTATSAD-ATTHIAITFFISAIHQIAL-	
PtIP-50Fr	(869)	EENKGLAFPTATSADAATTHIAITFFISAIHQVAL-	

Fig. 14a

	1		50
PtIP-50Ga	(1)	MHPRAHDGIS-IPLQGGDDHDAEERQISQMRPRFKAN-VVEDYEEIYRGS	
PtIP-50Gb	(1)	MHPTGHDGISNIPLQCHDDYAE---RQMKPRFETNGLELDYEKIYAGS	
PtIP-50Gc	(1)	MHPTGHDGIS-IPLQGGDDYAEES-RQMIRPRFKANGLELDYERIYAGS	
PtIP-50Gd	(1)	MQQSVHIAARMPGDGEKGRFSSDEEHQMMRPRLTSN-VPEDYEAIYAGS	
	51		100
PtIP-50Ga	(49)	NSIAREVGVSKVNCGEVMAIHRMFFRLDITLSQELLRQPERVQRLIVVADV	
PtIP-50Gb	(48)	NSIAREVGLDKVNCGEVMAIHQMFFRLDITLSQELLRPELVKRLIVVADV	
PtIP-50Gc	(49)	NSIAREVGLNKVICGEVMAIHRMFFRLDITLSQELLRQPELVKRLIVVADV	
PtIP-50Gd	(50)	NSITREVGVNKVRCEVMAIHQMFFRLDITLSQELLQOPQLVKHLIVVADV	
	101		150
PtIP-50Ga	(99)	VEIEGSNDITLSLPGSSLVLVLCRILILKSKDVVLDLTSWDLTVATTE-PL	
PtIP-50Gb	(98)	VEIEGSNATLSLPGSSLVLIFCRILILKSRNEVLDDLNNLRAASTT-PL	
PtIP-50Gc	(99)	VEIEGANATLSLPGSSLVLIFCRILILKSRDVVLDLSNWDLRVATTE-PL	
PtIP-50Gd	(100)	VEIEGSRGTLVLPGSSLVLIFCRILVLKSKDVVLDLGSWDLTVATTSPL	
	151		200
PtIP-50Ga	(148)	HRC SIHAQRVVVASG-SLSASLLIQVKHKFEWSDGTHLDQYFGRPFESFIA	
PtIP-50Gb	(147)	HRC SIRAQRVVVIASGFSLYSSLLIQVKHKFEWSDGTHLDQYFGRPFYFTA	
PtIP-50Gc	(148)	HRC SIRAQRVVVASG-SLSASLLIQVKHKFEWSDGTHLDQYFRRPISFTA	
PtIP-50Gd	(150)	VRC SIRAQQVVISSG-SLSASLLIQAKHRLRWSGSHLDQYFGRPFESFAS	

Fig. 14b

		201		250
PtIP-50Ga	(197)	SEINSSATATTTRENSAMRSISTWPGTAPVSNFIRVNSEKFNVLVNAVVPWK		
PtIP-50Gb	(197)	IGINSSATITTTATSAMRSINTWPGTAPVSNFIRVNSEKFNVLVNAVVPWK		
PtIP-50Gc	(197)	SRINSSAAITTRTTSATRSINTWPGTAPVSNFIQVNSEKFNVLVNAVVPWK		
PtIP-50Gd	(199)	SKINSFATTTTQTSYAKQSISTWPGVPPTS NLIVVNSETFNVRVNAVVPWK		
		251		300
PtIP-50Ga	(247)	GVVDCQASLPSLDDLLHPDVISGIQSTLLIVETILNFQTDNPAAIIILAQQ		
PtIP-50Gb	(247)	GVVDCQAALPSLDDLLHPDVISGFQSTLLIVETILNFQTDNPAAIISLARQ		
PtIP-50Gc	(247)	GVVDCQAALPSLDDLLHPDVISGIQSTLLIVETILNFQTDNPDIIIVLARQ		
PtIP-50Gd	(249)	GVVDCQTALPSSDDLLHPHVISGIQSTLLIVETILNFQTSNPSIIIVLAQQ		
		301		350
PtIP-50Ga	(297)	HAEWIVDSLLOVVHLPDSTFGVAEVKTL LARAQMLMKLPTDGSQHLQVPL		
PtIP-50Gb	(297)	HAEWILDSLLOVVLPDSTLGVPEAKTL LARAQILMKLPTDGSQHLLRVPL		
PtIP-50Gc	(297)	HAEWIVDSLLOVVLPNSTLGVPEVKTL LARAQMLIKLPTDGSQHLLRVPL		
PtIP-50Gd	(299)	HAQWIVDSLLOQAVHLPNSTTGVPELKTLLARSQMVVVKLPIDGSQHLKVPL		
		351		400
PtIP-50Ga	(347)	LAYGEYQEDIDQLLRNAEAYDQEYRELTRFVQVVEIIGNEFLQLSRSLAE		
PtIP-50Gb	(347)	LAYGEYQEDIERLLRNAEAYDQEYRELTRFVQLVEIIGSEFLQLSRSLAQ		
PtIP-50Gc	(347)	LAYGEYQEDIERLLRNAEAYDQEYRALTRFVQLVEIIGSEFLQLSKTLAQ		
PtIP-50Gd	(349)	LVYGEYQEDIDQLLRNAEAYDQEYRQLTRFVQVQVQIIGSEFLQLSMSLAQ		

Fig. 14c

		401		450
PtIP-50Ga	(397)	KERQIEIFESLVVIRKQSELDQAIRRMNSLMTEIERRSYEMADARSEMEQ		
PtIP-50Gb	(397)	RERDIEAFESLAVIRKQSELDQAIRRMNSLMPEIERRSFEMADAKARMEE		
PtIP-50Gc	(397)	RERDIETFESLVVIRKQSELDQAIRRMNSLMTEIERRSFEMADAKARMEE		
PtIP-50Gd	(399)	KERQIETFQSLLVIRKQSELDQTMRRIDSLMREIERRSFEMADARYMEE		
		451		500
PtIP-50Ga	(447)	GLEDYHRRQLTRAFGILGALLQLCASLKFGGGADIAGGIATTVPAAIDM		
PtIP-50Gb	(447)	GLEDYNRRQLTRAFGILGALLQLCASLKFGGGADIAGGIATTVPAAIDL		
PtIP-50Gc	(447)	GLQDYNRRQLTRAFGILGALLQLCASLKFGGGADIAGGIATTVPAAIDL		
PtIP-50Gd	(449)	GVLDPYRRQVNRVAVFGLGALLQLFASLKFGGGADIAGMETTVSAAIDI		
		501		550
PtIP-50Ga	(497)	IHAVEDASANTKLLAISQSLMDLEKIVDVVNAVVALVESATELEDIIHAP		
PtIP-50Gb	(497)	IHAVQDTSASTKLLAISQTLMDLEKIVDVVNAVNTLAESATELEDIVHAP		
PtIP-50Gc	(497)	IHAVQDDSASTKLLAISQTLMDLEKIVDVVNAVNELVESATELEDIIHAP		
PtIP-50Gd	(499)	IHAVEDASASSKLLPNTKNIIDLEKMEVVNAVNELVENATELEDIIHAP		
		551		600
PtIP-50Ga	(547)	ELPLIPPYTWDIENDIEEFAALMPSEVSEVVTKAKCRNLVAVCREICI		
PtIP-50Gb	(547)	ELPLIPPYTWDIENDIEEFAALMPLEVSEVVTKAKCRNLVAVCREICI		
PtIP-50Gc	(547)	ELPIILPYTWDIENDIEEFAALMPSEVSEVVTKAKCRNLVAVCREICI		
PtIP-50Gd	(549)	ELPIISSYTWDIENDIDELAAALMPSEVSEVVTKAKCRNLVAVCREICI		

Fig. 14d

	601		650
PtIP-50Ga	(597)	AASFACEVQYELFVHARQQEMARRQAERLEGMQIAADLSSYIELATQADM	
PtIP-50Gb	(597)	AASFACEVQYELFVHARQQEMARRQAERLEGMQTAADLSSYIELATQADM	
PtIP-50Gc	(597)	AASFACEVQYELFVHARQQEMARRQAERLEGMQTAADLSSFIELATQADM	
PtIP-50Gd	(599)	AANFAAEVQYELFVHARQQEMARRQAERLEGMQLATDLSSYIELATQADM	
	651		700
PtIP-50Ga	(647)	RTTRLLLSLLNVLAGHQGALHYHYLMELEVFTFSWPSVDSVRMHLQLNQ	
PtIP-50Gb	(647)	RTARLLLSLLNVLAGHQGALHYHYLMELEVFTSSWPTVDSVRTHLLQLNQ	
PtIP-50Gc	(647)	RTTRLLLSLLNVLAGHQGALHYHYLMELEVFTSSWPSVDGVRMHLQLNQ	
PtIP-50Gd	(649)	RTTRLLLSLLNVLADHQGALHYHYLMEIQVFTFTWPSVDSVRMHLRLNQ	
	701		750
PtIP-50Ga	(697)	RARARESGLEFNGVLTVDLEHNYVLD AIPV SLLSGEDYNFTINPERNAS	
PtIP-50Gb	(697)	RARARENALFGDGVLTVDLQHDYVLD AIPV SLLSGEDWNFTINPERNAS	
PtIP-50Gc	(697)	RARARENALFGDGVLTVDLQHDYVLD AIPV SLLSGEDWSFTINPERNAS	
PtIP-50Gd	(699)	WARARENMLFGPGVLTVEVQDYVVD AIPM SLLSGEDWSFTINPGONAS	
	751		800
PtIP-50Ga	(747)	SFPTPWDYVRIRYVEMKFTGEHQPVTKTGEIYLVLRSSANFQDRLEEQVF	
PtIP-50Gb	(747)	SFPTPWDYVRIRYVEMRFTGEHLPVSETGEIYLLRSSANFQDRLEEQVL	
PtIP-50Gc	(747)	AFPTPWDYVRIRYVEMKFTGEHLPVTD TGEIYLLRSSANFQDRLEEQVF	
PtIP-50Gd	(749)	VFPAPWDYVRIRYVEMKFTGEHHPVSQTGEVYLLQSSANFQDRFEGQVL	

Fig. 14e

		801		850
PtIP-50Ga	(797)	EYEA	AVPLVYQYAYNLSTGATTLPNLP	PSQSGKFFRMPFTRWRLRLSASA
PtIP-50Gb	(797)	EYEA	AVPLVYQYAYNLSTGATTLPNQ	PSESGKFFRMPFTRWRLRLSASA
PtIP-50Gc	(797)	EYEA	AVPLVYQYAYNLSTGETTLPNLP	PSESGKFFRMPFTRWRLRLSASA
PtIP-50Gd	(799)	EYEA	AVPLVYQYAYNLSTGATTLPNLP	PFESGKFFRMPFTRWRLRLSASA
		851		900
PtIP-50Ga	(847)	YQNE	GVSEPTLEDLPGAADSPVQITIT	TFYVTALPQIQTS-STDQDPASTT
PtIP-50Gb	(847)	YQNQ	GVSEPTLEPGDEN-SDSPVQITIT	TFYVTALPQIQSR-SLDMSDGDPT
PtIP-50Gc	(847)	YQNE	GVSEPTIPGDPTSADTPVRITIT	TFYVTALPQIQSRSLDMST----
PtIP-50Gd	(849)	YQNE	GISFPTANSSDI--S--IQITIT	TFYVTALPQIQSL-SEDPVAVATL
		901		
PtIP-50Ga	(896)	---		
PtIP-50Gb	(895)	EE-		
PtIP-50Gc	(893)	---		
PtIP-50Gd	(894)	---		

Fig. 15a

	1		50
PtIP-50Fa	(1)	MEYSALYGDVNQVSLRFEKTEFSEVVVHRMHVRLAELNVTLGDNNILQ	
PtIP-50Ff	(1)	MEYSSLYGDVNQVSLRFQNMETFSEVMVHRMHVRLLEELDMTG-----LE	
PtIP-50Fq	(1)	-----VNQVSLRFQNMETFSEVMVHRMHVRLLEELDMTG-----VE	
PtIP-50Fi	(1)	MEYSDLYHDVNMSLPLHRTELSEVMVHRMHVRLRDLNLET-----A	
PtIP-50Fj	(1)	MEYSDLYADVNOVSLRFQTEFSEVMVHRMHVRLLDLDMTTEPHLQMLA	
	51		100
PtIP-50Fa	(51)	GSDRVKRLYVLADVVELPP---IFSTSMSLPSTVSVVILCRILYLPYLED	
PtIP-50Ff	(45)	GIEKVKRLYVLADVVELPSTATQVFQYLRLPASISAILCRVLYIPEVDQ	
PtIP-50Fq	(36)	GIEKVKHLYVLADVVELPPKATSVFEYVRLPATISAILCRVLYIPEVQR	
PtIP-50Fi	(44)	VSDKVHRLYVVADVVEV-----TLDTVSLPCKVSVVILCRVLHVE--QS	
PtIP-50Fj	(51)	GSEKVRRLYVVADVVELP----ATATNMRLPATASVVILCRVLYVQDVNS	
	101		150
PtIP-50Fa	(98)	ASSASG---WAVHAALRLRVVEMHAGFAQVFTIPANDSSAAAPADIGLY	
PtIP-50Ff	(95)	RP---HMAQCSLDFFMRLHVVGSAH-ENGGGVMOAFSSD-ATPSNIGIY	
PtIP-50Fq	(86)	LSGAGFRGQCSLDFFMRLHVVGAAH-ESGGGVMOAFSSD-ATAGNIGVY	
PtIP-50Fi	(86)	SVLF-----SSLLENMNVRVVLSN---NRMQVYPMATDG--SADDVALY	
PtIP-50Fj	(97)	PYLA-----CGLHPLMPLRVVEDTQ-QGRVHVFPVVGESASVDKIGLY	
	151		200
PtIP-50Fa	(145)	IHADRIIYRDD-PGRSIPVIEVRVGGDSRFSPFLP---GSP--EIRLSSV	
PtIP-50Ff	(140)	FHADRFIYRQATSSASNFVLPDVRVSEFGSSTFSAPTLRPDWQNLNVSNI	
PtIP-50Fq	(134)	LHADRFIYREAASPASDFVWPLDVRVSEFGSSTFSN-TAVPDWQNLNVSII	
PtIP-50Fi	(126)	IHADRVIRRQG-AWTLVRVMHMSITGPE-----STFSVPHLPDPALFNRY	
PtIP-50Fj	(141)	IHADRVVYRQD-TSTANPLQPLQLRVIGNGSTFSTEVRPDWPKTLEVSSL	

Fig. 15b

		201		250
PtIP-50Fa	(189)	QYVREFSK-GQPVAPEESELQRSDEIETAP-----		SGSEQQLRA
PtIP-50Ff	(190)	QYGPQHLSRGPPETSSDSDLQRSDEIELLAQQDVWSPLLHVAFSPNAPPG		
PtIP-50Fq	(183)	RYDSQRLSKGPPETSSDSDLQRSDEIELLAQQDIWSPILHVSEFNPNAPPG		
PtIP-50Fi	(170)	MVNVQMDA-GPSPASESDLQRSDETELVS-----		RVSNQVTVSASIA
PtIP-50Fj	(190)	LMWLTLSK-GQPIPAESDLQRHDEVELLSPSPD--		RLSVVFAYLAPGN
		251		300
PtIP-50Fa	(228)	AFGASVGMNLLFHFSSFDILSLDPVPAALLTDPNIITGMQMNMLIAELV		
PtIP-50Ff	(240)	NIPGTQGLFRPSSACSEFHVPPPD-VPANVLTDPSTIILGMQMNMLIAELV		
PtIP-50Fq	(233)	NVPGTQGLFRPSSACSEFHVPPPD-VPANVLTDPSTIILGMQMNMLIAELV		
PtIP-50Fi	(212)	GSAWTMPADNPSATTFVFPNPPAQPVARGVLTNPYVIIGLQMNMLTAEV		
PtIP-50Fj	(237)	LFSIAVDVEYIGAGFCYFRIPPADPVETEVLTPYVIIGLQMNMLIAELV		
		301		350
PtIP-50Fa	(278)	LTAAHNSPOLIKVVRHVEWLNKMF-----		LQLASPNDLILALL
PtIP-50Ff	(289)	LAAHN-SPQVMNVTKHVLWLNKIL-----		LQVASPNDLILALL
PtIP-50Fq	(282)	LAAHN-SPQVMNVTKHVLWLNKIL-----		LQVASPNDLILALL
PtIP-50Fi	(262)	QAAHN-APLLIRAVTRHVEWLNKIL-----		VEALQVVSGNEDLILALL
PtIP-50Fj	(287)	LAAHN-SPPVISVTKHVEWLNKILPNDLILALLFRVQLSLPNDLILALL		
		351		400
PtIP-50Fa	(317)	FRVQAYQKMAKQP----		HPVVPRMRYSRYEGLINQMVQIAQSYDQDLKQL
PtIP-50Ff	(327)	FRIQAFMKMAKEP----		RFVVPRLOQHMYGSLINRMVQVAQNYDQEFKQL
PtIP-50Fq	(320)	FRIQAFMKMAKQPRFVPRFVVPRLOQHMYGSLINRMVQVAQNYDQEFKQL		
PtIP-50Fi	(303)	FRTETYLKMATES----		RSVVPRLOQHMYSDLIHRMVQVAQAYDDEFQRL
PtIP-50Fj	(336)	FRVQAFMKMAKQP----		RSVVPRLOQHMYSPINRMVQVAQVYDQEFKQL

Fig. 15c

	401		450
PtIP-50Fa	(363)	KLFIAQNEILGSYLLQNKAFAAKEKSMSEFHLQVSDLRRSELNDAIQKM	
PtIP-50Ff	(373)	KLFIAQNKILGSYLLQONRAFAEREREMSAFHSQVVSMMRRELQSAIQTM	
PtIP-50Fq	(370)	KLFIAQNEILGSYLLQONKAFAREKEMSAFHSQVVSMMRRELTTALETM	
PtIP-50Fi	(349)	RLFIAQNEILGSYLLQNKALASREREMSAFHSQVVSLLRRETELDNCLQRM	
PtIP-50Fj	(382)	KLFIVQNQILGSYLLQNKAFASRETEMSSFHSQVVSLLRRETELNNAIDRI	
	451		500
PtIP-50Fa	(413)	TGLGEEMEVEKEAMQAYKDMEQGLQEYKROFVTAVFAVISAIISLALG	
PtIP-50Ff	(423)	DNLSLQMESESEAMNEAQENMVEAIQEYERKLLARALFSVIGAIASVALA	
PtIP-50Fq	(420)	NQLSLQMETESEAMNEAQENMVEAIQEYERKLLARALFSVIGAIASVALA	
PtIP-50Fi	(399)	DQLNVQMERENKAMEEAQEKMNEALEAQRRLARALFAVLGAIAAVALT	
PtIP-50Fj	(432)	DQLSVQMENENEAMQAKEDMMQATAEYKQLANALFAVLGAIASVALA	
	501		550
PtIP-50Fa	(463)	FVTGGATVAAVPGAVANAAQAVSAAVSLANKLQKVMGILEIINQVVQTAA	
PtIP-50Ff	(473)	FATGG---ATAPGAVAAAGGAVAAAGRLAAGLQKVVVDILOGLQAVMEVVV	
PtIP-50Fq	(470)	VATGG---ATAPGAVAAARGAVTAAGRLAAGLQKVVVDILOGLQAVMEVVV	
PtIP-50Fi	(449)	VATGG---AAPAAVAAAGQAVTAAGTLAAGLKTVEILEGLQALMDLVV	
PtIP-50Fj	(482)	FATAG---ATAPGAVAAAGAAVSAAGRLAAGLKKVVEILEGLAAVMEIVA	
	551		600
PtIP-50Fa	(513)	AIKEVIELFDNMGQLLEAPEMPEMPSNYDWLIFVNEVEAMAEQIPVEVN-	
PtIP-50Ff	(520)	AIRDIVESLKNMGQLVEAPEMPEMPTDADWLIFVNEVEAVAEQVPTEVA-	
PtIP-50Fq	(517)	AIRDIVESLKNMGQLVEAPEMPEMPTDADWLIFVNEVEAVAEQVPTEVA-	
PtIP-50Fi	(496)	IIRELVENLQITIGQLIDAPEMQEMPSNADWLIFVNEVEAVAAQMPPEVFG	
PtIP-50Fj	(529)	AIRDLVESLQNLGQLVETPEMPDMPNADWLIFVNEVEAVAEQMPAEVVG	

Fig. 15d

		601		650
PtIP-50Fa	(562)	ERIVWKAKCKNVAVLGQEMCTTGSHIADLQYQIKVEEMLREIAQSQAEERL		
PtIP-50Ff	(569)	EVPVWKAKCKNVAVLGQAMCTTAAYISELQYQITVEEMLQEIAQRQADRL		
PtIP-50Fq	(566)	EVPVWKAKCKNVAVLGQAMCTTAAYMSELQYQITVEEMLQEIAQRQADRL		
PtIP-50Fi	(546)	SVAVWKAKCRNVAVLGQEMCTMAAHIAELQQQIKLEELLQEIADRQADRL		
PtIP-50Fj	(579)	SVAVWKAKCRNVAVLGQEMCTTAAHIGELTYQIKVEEMLQEIAQRQANRL		
		651		700
PtIP-50Fa	(612)	EGISSADLSSYSTEMVSQIDMRTTRLLFQLIKVLHIQNAALKYEYLYAADE		
PtIP-50Ff	(619)	VGISAADLSSYSTEMASQIDMRTTRILLELIKMLYIQNAAIKYEYLYDANE		
PtIP-50Fq	(616)	VGISAADLSSYSTEMASQIDMRTTRILLELIKMLYIQNAAIKYEYLYDANE		
PtIP-50Fi	(596)	LGISAADLSSFTEMLTQIDMRTTRLLLQLIKLHIQNVAINYEYLYPANG		
PtIP-50Fj	(629)	ESLTPANLSSYSTEMSEIDMRTTRLLLQLIRELHIQNAAIKYEYLYAAGE		
		701		750
PtIP-50Fa	(662)	HLVSWPVSMETVWEMLLQQQRLSLLGWENLVGINNLPNDDTRTYVVKDIP		
PtIP-50Ff	(669)	KLNSWPVSMETVWEMLLQQENAALLGLLDLGPIN----DFTVITYAVKDIP		
PtIP-50Fq	(666)	KLNSWPVSMETVWEMLLRQENAALLGLLNLGPSN----DFTVITYAVKDIP		
PtIP-50Fi	(646)	RLSSWPVTMHTVWEMLLQQESSAITGLLALGPST----DFARTFVVHDIP		
PtIP-50Fj	(679)	QLNSWPVSMETVWEMLLQQENSALVGLLDLGPSN----DFTRSFVIQGIP		
		751		800
PtIP-50Fa	(712)	VEVLLSGRDWPFVIHEEDYT-AFPSGWYYVRINVELKFEQQTAGSTDIV		
PtIP-50Ff	(715)	TKLLVDGFDWNFIEIATEDFA-IFPSGLSRVRIRYVELKFDQQGADSSNIV		
PtIP-50Fq	(712)	VKLLVDGYDWNFEIATEDFS-IFPSGHSRVRIRYVELKFDQQGTDSSNIV		
PtIP-50Fi	(692)	VGLLVDGEDWQFAIPVMDSSAFPIGFSRVRIRHVELKFSALIDIDAS-AT		
PtIP-50Fj	(725)	IGLLVDGNDWQFSIPVEDSP-TFPIGYSRVRIRYVELRFDQGTSDSEG-RM		

Fig. 15e

		801		850
PtIP-50Fa	(761)	IHQ	STNTGRLYMLLQSSRFFHDRNQGVKMEYEGSRGLSPYAYNLNTGE	
PtIP-50Ff	(764)	IHQ	STNTGLVYMLLQGSRFVHDRKROEVMDYEASTGPVYAYAYDLNTGA	
PtIP-50Fq	(761)	IHQ	STNTGLVYMLLQGSRFLHDRKREEVMDYEASMGAVFAYAYDLNTGA	
PtIP-50Fi	(741)	VHQ	PRTDNGFTYLLQSSARSFSDRRVRQAMDYEASEGLAYPYAYNLTTGV	
PtIP-50Fj	(773)	IHQ	STSSGLTYMLLQSSPVFRDRRRREVLEYEASMGLAYARAYNLTTGV	
		851		900
PtIP-50Fa	(811)	TTHNNIPSDQFKNIFMQMTPFT-TWRLRLSASAEENGLVFHPPSTSPTS		
PtIP-50Ff	(814)	TTLNNIPSQQQANTFMQMPFN-AWRLRLSASAAENQGLVFP-TATSPDN		
PtIP-50Fq	(811)	TTLNIPSQQHANTFMQMPFNAAWRLRLSASAMENQGLVFP-TATSPDN		
PtIP-50Fi	(791)	PSLTNLP SQEHANTFMRMTPFT-EWRLRLSSSAEENRGLTFP-TATSPDD		
PtIP-50Fj	(823)	PTLTNVPSPEFANTFMRMTPFN SNWRLRLSSSAMENQGLMFP-TASSADD		
		901		934
PtIP-50Fa	(860)	TTQVSITFHVTRIRRIDRFTSTVHQKHAS-----		
PtIP-50Ff	(862)	TTQISITFYVTAIRRIDLRQEGDVE-----		
PtIP-50Fq	(860)	TTQISITFYVTAIRRIDHRQEGDEE-----		
PtIP-50Fi	(839)	STQISITFHITAIRAIDFRGMDDEERSKSDSSQK		
PtIP-50Fj	(872)	TTQITITFFISAIRGIDTRAAVEI-----		

Fig. 16b

```

[ motif 23 ][          motif 8          ]
          60          *          80          *          100
IPD050Fb : AVPDYSHLYRELNQVSEGMKLDQMEFSEVMVIHRMFIRLHDLNIAH---- : 49
IPD050Fs : AVPDYSHMYRELNQVSEMRRLDHFSEVMVIHRMFIRLPDLIDIAL---- : 49
IPD050Fo : AVPDYSHMYRELNQVSEMRRLDHMEFSEVMVIHRMFIRLPDLIDIAL---- : 92
IPD050Fk : AVPDYSHLYRELNQVSEIRRLDQMEFSEVMVIHRMFIRLPDLNIAH---- : 96
IPD050Fe : AVPDYSHMYRELNQVSEIRIMDQMEFSEVMVIHRMFIRLPDLSIAH---- : 49
IPD050Fl : AIPDYSNLYRELNQVSEMRMDQMEFSEVMVIHRMFFKLPDLIDIAH---- : 49
IPD050Fn : AIPDYSHLYRELNQVSEIRIMDQMEFSEVMVIHRMFIRKFPDLIGIAH---- : 49
IPD050Ft : AAREYSELYKDLNQISERIRLDQMEFSEVMVIHRMFIRLEELDIGH---- : 49
IPD050Fh : AGADWRELYSEMNVQSERLRRLDQLEFSEVMVIHRMHIKLSELDLGH---- : 53
IPD050Fg : GIVDWRELYRDMNQASDRIRFNQLEFSEVMVIYRMHIKMSSELDMGH---- : 53
IPD050Fm : AADAWREVYRDLNQVSDRIRLDQLEFSEVMVIHRMHIKMSSELDLGH---- : 59
IPD050Fd : ANEAWREVYHDLNQVSDRIRLDQLEFSEVMVHRMHIKMSSELDLGH---- : 54
IPD050Fr : AADGWREVYRGLNQVSDRIRFDQLEFSEVMVIHRMHIKMSSELDLGH---- : 53
IPD050Bb : ADIDYSVLYNDVNQIS--IRLERMDFSEVMVHRMFVRMDDLVDVSSGTG- : 48
IPD050Bd : AGIDYSVLYQDVNQIS--IRLEKMDFSEVMVHRMFVRMDDLVDVSSGTG- : 48
IPD050Aa : AAGDYSVLYQDVNQIS--IRLEKMDFSEVMVHRMFVRMDDLVDVSSGTG- : 48
IPD050Ba : ADLDYSKLYQDLNQIS--VRLEKTEFSEVMVHRMFVRMDDLVDVSSGSG- : 48
IPD050Bc : ADVDYSKLYHDLNQIS--IRLGNVEFSEVMVHRMFVRMDDLVDVSSGSG- : 48
IPD050Fa : --MEYSALYGDVNQVS--LRFKTEFSEVVVHRMHVRLAELNVTLGDNN : 46
IPD050Ff : --MEYSSLYGDVNQVS--LRFQNEFSEVMVHRMHVRLLEELDMTG---- : 42
IPD050Fq : -----VNQVS--LRFQNEFSEVMVHRMHVRLLEELDMTG---- : 33
IPD050Fi : --MEYSDLYHDVNMLS--LPLHRTELSEVMVHRMHVRLDLINLET---- : 42
IPD050Fj : --MEYSDLYADVQVS--LRFQTEFSEVMVHRMYVRLDLDMTTEPHL : 46
IPD050Fp : SLPDYEAIYKVNQVS--RPVASRQASEVTSIHRMYFKLNLLLEVAL---- : 46
IPD050Gd : VPEDYEAITYAGSNSITREVGVNKVRGELMAIHQMFFRLDLSQEL---- : 83
IPD050Ga : VVEDYEEIYRGSNSIAREVGVSKVNCGEVMAIHRMFFRLDLSQEL---- : 82
IPD050Gb : LEDDYEKIYAGSNSIAREVGLDKVNCGEVMAIHQMFFRLDLSQEL---- : 81
IPD050Gc : LEDDYERIYAGSNSIAREVGLNKVICGEVMAIHRMFFRLDLSQEL---- : 82
IPD050Fb : CCCCCNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNNNNNCCCCC---- : 49
IPD050Fs : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 49
IPD050Fo : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 92
IPD050Fk : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 96
IPD050Fe : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 49
IPD050Fl : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 49
IPD050Fn : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 49
IPD050Ft : NNNNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 49
IPD050Fh : CCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 53
IPD050Fg : CCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 53
IPD050Fm : NNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 59
IPD050Fd : CCCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 54
IPD050Fr : CCCCNNNNNNNNNNNNNNCCCCCCCCNNNNNNNNNNNNCCCCC---- : 53
IPD050Bb : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 48
IPD050Bd : CCCCNNNNNNNNNNCC--CANNCCNNNNNNNNNNNNCCCCC---- : 48
IPD050Aa : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 48
IPD050Ba : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 48
IPD050Bc : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 48
IPD050Fa : --CCCCCCCCNNNN--NNNNCCCCCCCCNNNNNNNNNNCCCCC---- : 46
IPD050Ff : --CCCCCCCCNNNN--NNNNCCCCCCCCNNNNNNNNNNCCCCC---- : 42
IPD050Fq : -----CCCC--NNNNCCCCCCCCNNNNNNNNNNCCCCC---- : 33
IPD050Fi : --CCCCCCCCNNNN--NNNNCCCCCCCCNNNNNNNNNNCCCCC---- : 42
IPD050Fj : --CCCCCCCCNNNN--NNNNCCCCCCCCNNNNNNNNNNCCCCC---- : 46
IPD050Fp : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 46
IPD050Gd : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 83
IPD050Ga : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 82
IPD050Gb : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 81
IPD050Gc : CCCCNNNNNNNNNNCC--CCCCCCCCNNNNNNNNNNNNCCCCC---- : 82

```


Fig. 16d

```

-----]
                160          *          180          *          200
IPD050Fb : LSVRSF-----QSQHGLDFPHMRLHAV-PGDNQMLRIHR : 122
IPD050Fs : LLFQGS-----YSAYDLDFPHMRLHAV-PGEAQILRIHR : 129
IPD050Fo : LFYQG-----FARYDLDFPHMRLHAVQPSGAGVLRMHR : 173
IPD050Fk : LSLRSP-----TARYGLNFPHMRLHAVFQSASRILHMHR : 170
IPD050Fe : LTLHVDPI-----ATAYQCDFPHMRLHAFFGSNG--VRIDR : 123
IPD050F1 : LTLHADWS-----RTSYRFVFPHMRLHAIFDLQQ--ARIHR : 123
IPD050Fn : LTLHLDSA-----AQAYRIDLPHMRLHAINDAQR--LRIHR : 123
IPD050Ft : LSVRVLME-----DNGNNAMRLQLPHMRLRVVEITTOPTTKLQI : 134
IPD050Fh : WHVSRRLTVGVNSPQYWLPFTFDGRPVRIQLPHVKWHMNTLTRS----MHR : 144
IPD050Fg : WHVSYHLVARMDRNP PRYQLDLAQGVIISLPHMAWHL D VRRQS----MHR : 138
IPD050Fm : WHINYALVQISS-----FARISRSVVLELRHMRWHFNAITGR----MHL : 147
IPD050Fd : WHINVGVVQLTS-----TVQMSRTLIVQLPHMRWHVNAT TGS----MHR : 142
IPD050Fr : WHVNYGIVQRTS-----TVRVARTVVVQLPHMRLHFNAT TGS----MHR : 141
IPD050Bb : FVRNGRHSTEL-----FLPSMNMSMVAA-GNGTIRGVN : 119
IPD050Bd : FVVNGRHSTEL-----FLPSMNLNMVAA-GTGSIRGAY : 119
IPD050Aa : FVTNGRHSTEL-----FLPSMNMSMVAP-GTGSIRGVI : 119
IPD050Ba : FVPEGRHYAEL-----FLPSMNMSMVGADGEGSIRGVI : 120
IPD050Bc : FVINGHHGTEL-----FLPSTSMSVAA-GEGSIRGVI : 119
IPD050Fa : LYLPYLEDASS-----ASGWAVHAALLRLRVVEMHAG-FAQVFT : 127
IPD050Ff : LYIPEVDQRP-----HMAQCSDLDFPFMRLHVVGSAHENG G GVMQ : 125
IPD050Fq : LYIPEVQRLSGAG-----FRGQCSDLDFPFMRLHVVGAAHESGG GVMQ : 119
IPD050Fi : LHVE--QSSVL-----FSSLLLPMNVRVLSNN--RMQVYP : 112
IPD050Fj : LYVQDVNSPYL-----ACGLHFPLMPLRVVEDTQQGRVHVFP : 125
IPD050Fp : LSLKRED-----VELGFKFLGPVECEEGWQVGLNS : 114
IPD050Gd : LVLKSKD-----VVLDLGSWDL SVATTS S PLVRC SIR : 155
IPD050Ga : LILKSKD-----VVLDLTSWDLTVATTT-PLHRCSIH : 153
IPD050Gb : LILKSRN-----LVLDLDNWNLRAASTT-PLHRCSIR : 152
IPD050Gc : LILKSRD-----VVLDLSNWDLRVATTT-PLHRCSIR : 153
IPD050Fb : HCCCC-----CCCCCCCCCCCCCCCC-CCCCCEEECC : 122
IPD050Fs : HHCCCC-----CCCCCCCCCCCCCCCC-CCCCHHHHHCC : 129
IPD050Fo : HHHCC-----CCCCCCCCCEEEECCEEEECCEEEEC : 173
IPD050Fk : HCCCC-----CCCCCCCCCHHHHHHHHHHHHHHCC : 170
IPD050Fe : EEECCCC-----CCCCCCCCCHHHCCCCC--EECC : 123
IPD050F1 : HCCCC-----CCCEEECCCHHHHCHHH--HHHC : 123
IPD050Fn : HHHCCCH-----HHHHCCCCCCCCCCCCCH--HHCC : 123
IPD050Ft : EEEEEEE-----ECCCCCECCCCCCCCCEEEECCEEEEC : 134
IPD050Fh : EEECEEEECCEEEECCEEEECCEEEECCEEEECCHHHHHHHH----HCC : 144
IPD050Fg : CCHHHHHHCCCCCCCCCCCCCEEEECCEEEECCHHHCCCC----CCC : 138
IPD050Fm : EEECHHHHCH-----HHHHHHHHHHHCCCCCCCCCCCC----CCC : 147
IPD050Fd : EEEEEEEEE-----EEEECEEEECCEEEECCEEEECCEEEEC : 142
IPD050Fr : EEEEEEEEE-----EEEEEEEEECEEEECCEEEECCEEEEC : 141
IPD050Bb : HHHCCCC-----CCCCCEEEEC-CCCCCCCC : 119
IPD050Bd : EEECCCC-----CCCCCCCC-CCCCCCCC : 119
IPD050Aa : HHCCCC-----CCCCCCCC-CCCCCCCC : 119
IPD050Ba : EEECCCC-----CCCCCCCCCCCCCCCC : 120
IPD050Bc : EEECCCC-----CCCCCEEEEC-CCCCEEEE : 119
IPD050Fa : EEECCCC-----CCCHHHHHHHHHHHHCC-CEEEEE : 127
IPD050Ff : HCCCC-----CCCCCCCCCEEEECCEEEECCEEEEC : 125
IPD050Fq : HCCCC-----CCCCCCCCCEEEECCEEEECCEEEEC : 119
IPD050Fi : EEEE--CCHH-----HHCCCCCEEEEEEC--CEEEEE : 112
IPD050Fj : EEECCCC-----CCCCCCCCCEEEECCEEEECCEEEEC : 125
IPD050Fp : HCCCC-----CCCCCEEEECCEEEECCEEEEC : 114
IPD050Gd : HEECCC-----EEEECCCCCCCCCEEEEC : 155
IPD050Ga : EEECCC-----EEEECCCCCEEEEC-CCCCCE : 153
IPD050Gb : HHHCCC-----CCCCCCCCCCCCCCCC-CCCCCCC : 152
IPD050Gc : HHCCCC-----EEEECCCCCCCCCCCC-CCCCCCC : 153

```

Fig. 16e

```

*           220           *           240           *
IPD050Fb : RNPDSAQYDDYPVVCVHADVLEFPVTG-----GRDIFHLGPLANPC : 163
IPD050Fs : RNPDSAQYDDYPVVCVHADVLEFPVTG-----GRDIFHLGPLANPC : 170
IPD050Fo : LNPDSVQYDDYPVVCVLADVVEFTRQR-----PSRLFHIGPLAYPS : 214
IPD050Fk : LIPDSAQYDAYPVVCVHADALEFSGTS-----TGGIFHIGPLRYPS : 211
IPD050Fe : RNSDSVH-DDYPVITVSANVIEFSGTR-----AETLFAIAPLRDPS : 163
IPD050F1 : RNSDSVH-DDYPVITISANVIEFSGTRS-----SSVLFGIGVFLDPS : 164
IPD050Fn : RNSDSLH-DDYPVITISANVIEFSGTRS-----LRDIFGIGPLQDPS : 164
IPD050Ft : HRADAED-YQYPALCIHADVIQASIRLSSSSNRPFMDLRFNFRPNRGS : 183
IPD050Fh : IDPETLQDLGPELLCVYADLLRSLHPPVGHSNWDAALAFPSMTVLEVE : 194
IPD050Fg : LDPETLQEFLEPSFCVYADDISVAYYSINGAS-WDPALQFPDTAELAKCA : 187
IPD050Fm : LDPQTLQDGMDFCFCVHAEVVQIAYY-----PDVTFPESNDSPSFL : 188
IPD050Fd : LHPQTSQEGPDTSFVCVYAEVVQVAYY-----PDVTFSEFDGSPT-L : 182
IPD050Fr : LHPQTSQDGSDFCFCVYAEVVQIAYY-----PDVTYPEPESNDSPVGL : 182
IPD050Bb : LS-TTMSSSSSNALQFNLRSGSMTS-----TVRLKDVVDVAA : 154
IPD050Bd : LS-TTAFSLSSNALQFKLQSGSMTS-----AIQLKDVDLAA : 154
IPD050Aa : LSPTTTLTSSDALQFKLQSGSMTS-----VMRLKDVSVAA : 155
IPD050Ba : LS-PTVLTTLNLSNALQFRLECGSMTS-----VMRLNDVVSAGA : 155
IPD050Bc : LS-PTTLTFLSHALQFKLESGSMTS-----VMRLNDVSVAA : 154
IPD050Fa : IPANDSSAAAPADIGLYIHADRIYRDDPGRS-----IPVIEVRVGG-DS : 171
IPD050Ff : AFSSD---ATPSNIGLYFHADRFIYRQATSSASN--FVLPLDVRVSVFGSS : 170
IPD050Fq : AFSSD---ATAGNIGVYLHADRFIYREAASPAD--FVWPLDVRVSVFGSS : 164
IPD050Fi : MATDG---SADDVALYIHADRVIIRQGAWTL---VRVMHMSITGPES : 153
IPD050Fj : VVGES--ASVDKIGLYIHADRVIYRQDTSTAN---PLQPLQLRVIGNES : 170
IPD050Fp : EMAYIRRTSPQVRLSIYAQEVVHNSTG-----AILRVTHPVTVR : 153
IPD050Gd : AQQVVISSG-SLSASLLIQAKHRLRWSDGSHLDQYFGRPFSPASSKINSF : 204
IPD050Ga : AQRVVVASG-SLSASLLIQVKKHFEWSDGTHLDQYFGRPFSPFIASEINSS : 202
IPD050Gb : AQRVVIASGFSLYSSLLIQVKKHFEWSDGTHLDQYFGRPFYFTAIGINSS : 202
IPD050Gc : AQRVVVASG-SLSASLLIQVKKHFEWSDGTHLDQYFRRPISFTASRINSS : 202
IPD050Fb : CCCCCCCCCCEEEEEEEEECCCC-----CCCCCCCCCCCCCE : 163
IPD050Fs : CCCCCCCCCCEEEEECCCCCCCC-----CCCCCCCCCCCCCE : 170
IPD050Fo : CCCCCCCCCCEEEEEHHHHHHCCC-----CCCCECCCCCCCC : 214
IPD050Fk : CCCCCCCCCCEEEEECCCCCECCCC-----CCCECCCCCCCC : 211
IPD050Fe : CCCCCCC-CCCCEEEEEEEEECCCC-----CCEEEEECCCCCCC : 163
IPD050F1 : CCCCCCC-CCCCEEEEEEEEECCCC-----EEEECCCCCECCCC : 164
IPD050Fn : CCCCCCC-CCCCEEEEEEEEECCCC-----CCCCCCCCCCCC : 164
IPD050Ft : ECCCCCC-CCCCHHHHHHHHHHHCCCCCCCCCCCCCECCCCCCCC : 183
IPD050Fh : CCCCCCCCCCHHHHHHHHHHHCCCCCCCCCCCCCCCCCCCCCECC : 194
IPD050Fg : CCHHHHHHHCCCCEEEEECCCCCCCC-CCCCCCCCCHHHHHHHE : 187
IPD050Fm : CCCCCCCCCCEEEEEEEEEEECC-----CCCCCCCCCCCC : 188
IPD050Fd : CCCCCCCCCCEEEEEEEHHCCC-----CCCCCCCCCCCC-E : 182
IPD050Fr : CCCCCCCCCCEEEEEHHHHHCC-----CCCCCCCCCCCCCE : 182
IPD050Bb : CC-EECCCCCCCCCEEEEECCCC-----EEEECCCC : 154
IPD050Bd : CC-EECCCCCCCCCECCCC-----CECCCCCCCC : 154
IPD050Aa : CCCCEEEEECCCCCCCCCEEE-----EEEECCCC : 155
IPD050Ba : CC-CCHHCCCCCCCCCEEE-----EEEECCCC : 155
IPD050Bc : EE-CCCCCCCCCEEEEECCCC-----EECCCC : 154
IPD050Fa : EECCCCCCCCCCCCCEEEEEEECCCC-----CCCEEEEC-CC : 171
IPD050Ff : ECCCC---CCCCCEEEECCHHHHHHHCCCC-----CEEEEEEEEC : 170
IPD050Fq : ECCCC---CCCCCEEEECCHHHHHHHCCCC-----CCCCCEEEEC : 164
IPD050Fi : CCCCC---CCCCEEEEECHHHHHHCCCE-----EECCCCCCCC : 153
IPD050Fj : ECCCC---CCCCEEEEECEEEECCECCCC---CCCCCEEEEC : 170
IPD050Fp : CHHHHHHCCCCCEEEEEEECCCC-----EEEEECCEEE : 153
IPD050Gd : CCHHHHCC-CCHHHHHHHHHHHCCCCCCCCCCCCCCCCCEEE : 204
IPD050Ga : EEEEEEC-CEEEEEHHCCCCCCCCCCCCCCCCCEEE : 202
IPD050Gb : CEEEEECCHHHHHHHHHHHCCCCCCCCCCCCCCCCCEEE : 202
IPD050Gc : CEEEEEC-CCCCHHHHHHCCCECCCCCCCCCCCCCCCC : 202
```


Fig. 16f

```

                260          *          280          *          300
IPD050Fb : MVIT--FPPQS--LEEAPVS-----SFTGSQLWTAASS : 194
IPD050Fs : MVVT--HPQLS--LVEAPVS-----SFTGSQLWTAASS : 201
IPD050Fo : MIFTDERPPASSIVEPRFVS-----SFYCTIEFR---WSS : 246
IPD050Fk : MIVT---SESHSAVDAQIS-----SFSCNVSLRSASASS : 242
IPD050Fe : MIIS---SDLISNTVERPAS-----GFFCNVVENWG-QPS : 194
IPD050Fl : MVLS---SDRHSIVERPSS-----HTLCNVNPE--QSS : 193
IPD050Fn : MIITN--TETQPPVVERPSI-----NSLCTVGLVQGQSS : 197
IPD050Ft : NVVFS---SESRSLIDEFVE-----IFVYVSHI PRFIHAL : 215
IPD050Fh : VLPCFDHSTPRQG-LLTLTSNAAPQWVN--QLRAAFIFTDQDAMTEILSL : 241
IPD050Fg : LLIYSNSNPTPNHVLLTSNRFNGTRWAFRFEFSMY- IDNEDVAKDIALQ : 236
IPD050Fm : RFLRLEIVPDQ---LRVTTQTGDPVWALRGALYFMAPDNDGLLNEVSSQ : 235
IPD050Fd : RFLRLEFESDQ---LRVTTQTEGPIWAIASLDTTMTPDNDGLLNEISAQ : 229
IPD050Fr : RFLRLEFGSDQ---LRVTRQSEGPVWALRGLNYSMSPDNDGLMNEVSSQ : 229
IPD050Bb : ALTC-----DVQAASAS-----MPLTVMTTG---TSPGN : 180
IPD050Bd : TLTC-----DVQAASAS-----MPLTVMTTG---TSPGN : 180
IPD050Aa : TLTC-----NVQAASAS-----MPLTVMTTG---TSPGN : 181
IPD050Ba : TLTC-----NVQAASAC-----VPLKVKTTG---TSPGN : 181
IPD050Bc : TLAC-----NVQAASAS-----MPLKVKTTG---TSPGN : 180
IPD050Fa : RFSP--FLPGSPEIRLSSVQ-----YVR-EFSKQFPVAPEDS : 205
IPD050Ff : TFSAPTLRPDQNLNVSNIQ-----YGPQHLSRGPPLTSSDS : 207
IPD050Fq : TFSN-TAVPDWQNLNVSSIR-----YDSQRLSKGPPLTSSDS : 200
IPD050Fi : TFS---VPHLPDPALFNRV-----MVNVQMDAGPSIPASES : 186
IPD050Fj : TFST-EVRPDWPKTLEVSSL-----LMWLTLSKQQPIPAAES : 206
IPD050Fp : QRVS-----PHRPSQ-----YTPGMTKWQINISSA : 178
IPD050Gd : ATTTTQTSYAKQISITWPGVPP-----TSNLIVNSETFNVRVN : 243
IPD050Ga : ATATTRTNSAMRSISTWPGTAP-----VSNFIRVNSEKFNVLVN : 241
IPD050Gb : ATITTTATSAMRSINTWPGTAP-----VSNFIRVNSEKFNVLVN : 241
IPD050Gc : AAITTRTTSATRSINTWPGTAP-----VSNFIQVNSEKFNVLVN : 241
IPD050Fb : EEEE--ECCC--CCCCCCC-----CCCCCEEEEEEECCC : 194
IPD050Fs : EEEE--CCCC--CCCCCCC-----CCCCCEEEEEEECCC : 201
IPD050Fo : CCCCCCCCCCCCCCCCCC-----CCCEEEEEEE--EEC : 246
IPD050Fk : CCCC---CCCCCCCCCCCC-----CEEEEEEECCCCCCC : 242
IPD050Fe : CEEE---CCCCCCCCCCCC-----CCCCCCCCCCC-CCC : 194
IPD050Fl : CEEE---ECCCCCECCCC-----CCEEEEECCCC-CCC : 193
IPD050Fn : EEEEE--CCCCCECCCC-----CCCEEEEEEECCCC : 197
IPD050Ft : CEEEC--CCCCCCCCCEE-----EEEEEECCCCCCCC : 215
IPD050Fh : ECCCCCCCCCCC-CEEECCCCCHHHH--HHCCEEEEECCCCCCCC : 241
IPD050Fg : EEECCCCCCCCCEEECCCCCCCCCEEEEEEEEEEE--ECCCCHHHCCCC : 236
IPD050Fm : CEEEEECCCC--EEEEEECCCCCHHHCCCCCCCCCCCCCCCCCCCC : 235
IPD050Fd : EEEEEEEEEEE--EEEECCCCCHHHHCCCCCCCCCCCCCCCCCECCC : 229
IPD050Fr : EEEEEEECCC--EEEECCCCCCCCCEEEEECCCCCCCCCCCCCCCC : 229
IPD050Bb : CCCC-----EECCCC-----CCEEECCC--CCCC : 180
IPD050Bd : CCCC-----CCCCCCC-----CCEEECCC--CCCC : 180
IPD050Aa : CEEE-----EECCCC-----CCCCCCCC--CCCC : 181
IPD050Ba : EEEE-----EECCCC-----CCCCCCCC--CCCC : 181
IPD050Bc : CCCC-----CCCCCCC-----CCCCCCCC--CCCC : 180
IPD050Fa : CCCC-CCCCCCCCCCCCCHH-----HHH-HHCCCCCCCCCCCC : 205
IPD050Ff : CCCCCCCCCCCCCCCCCC-----CCCCCCCCCCCCCCCC : 207
IPD050Fq : CCCC-CCCCCCCCCCCCCEE-----ECCCCCCCCCCCCCCCC : 200
IPD050Fi : CCC---CCCCCCCCCCCC-----EEEEEECCCCCCCCCCCC : 186
IPD050Fj : CCCC-CCCCCCCCCHHHHH-----HHHHHCCCCCCCCCCCC : 206
IPD050Fp : EEEC-----CCCCC-----CCCCCEEEEEEEEC : 178
IPD050Gd : ECCCCCCCCCCCCCCCCC-----CCCCCCCCCCCCCEEC : 243
IPD050Ga : CEECCCCCCCCCEEECCCCCCC-----CCEEEEECCCCCEEE : 241
IPD050Gb : CEEEEEECCCCCCCCCCCC-----CCEEECCCCCCCCCCCC : 241
IPD050Gc : CEEEECCCCCEEECCCCCCCC-----CCCCCCCCCCCCCCCC : 241

```

Fig. 16g

```

*           320           *           340           *
IPD050Fb : PH---RIDPNNLTFT---PGFMVQQGGSFNLPFNTQSE----DFWLL : 231
IPD050Fs : PH---SIDPNNLTFT---PSFSVQGGGSFNLSFATRSE----DFWLL : 238
IPD050Fo : SN---NIGPNSVGIG---CVFRLSGGSTFST--GSPRE----DFWLF : 281
IPD050Fk : PL---KINPDNVSFQ---SMFNLVGTGGGFGF--AVRPE----EFWLL : 277
IPD050Fe : PI---TVDPLRIRFQ---TGATMVGGGSLT--FPLGPE----GFSIL : 229
IPD050F1 : PI---MVDPDTIRFQ---SIFNWVGSNIMSPQVARAPE----GFSLF : 230
IPD050Fn : PV---TVDPDRISRH---FWFQSVGGGALG--TLRAAE----GFSLF : 232
IPD050Ft : PIPEAATVDPASRSND---WYYVSLRRVNSTLSSSVAEEGELEPAFSL : 261
IPD050Fh : PADASVILNKNRPSII---LTAGQSGGYT-----DRNVSAPAFSVL : 279
IPD050Fg : PPGTRVIFKREGLHLV---VLAMDANRIISFDPERGDLDAYMPAFSLL : 282
IPD050Fm : PAGTSFVLNLPDNL---VQLFANLRPNSTLTR-DPNTIVPAFSVY : 280
IPD050Fd : PAGTIFKLNLPDNL---VSLFGLRPNSTSLTR-VPDNLPAFSIF : 274
IPD050Fr : PAGTSFILNLPDNLN---LQLMASLRNNSNSTLTR-DPDNIVPAFSVY : 274
IPD050Bb : IWLGMTAVVIPESA----- : 196
IPD050Bd : ILVLGMSTAVVIPESA----- : 196
IPD050Aa : ICVLGMSTAVVVPESA----- : 197
IPD050Ba : ICVLGLSTAAVVPESV----- : 197
IPD050Bc : ICVLGMSTDAVVPENV----- : 196
IPD050Fa : ELQRSDEIELIAPSGS-----EQQLRAAFGASVGMNLLFHFSSSFDIL : 248
IPD050Ff : DLQRSDEIELLAQQDVWSPLLHVAFSPNAPPGNIPGTQGIFRPSSACSFF : 257
IPD050Fq : DLQRSDEIELLAQQDIWSPILHVSNPNAPPGNVPGTQGLFRPSSACSFF : 250
IPD050Fi : DLQRSDETELVSRSVSN---GVTVSASIAGSAWMPADNP---SATFTVF : 229
IPD050Fj : DLQRHDEVELLSPSPDRLSVVFAYLAPGNLFSIAVDVEYI--GAGFCYF : 254
IPD050Fp : HADLSTAQRSHELRTQR-----FWSEPRTLWLNQEWSP-----FTTF : 217
IPD050Gd : AVPWKGVVDCQTALPS----- : 259
IPD050Ga : AVPWKGVVDCQASLPS----- : 257
IPD050Gb : AVPWKGVIDCQAALPS----- : 257
IPD050Gc : AVPWKGVVDCQAALPS----- : 257
IPD050Fb : CC---CCCCCCCC---CCEEECCCCCCCCCCCCCH---HHHHH : 231
IPD050Fs : CE---ECCCCCCCC---CCCCCCCCEEEEEEEECC---CCCHH : 238
IPD050Fo : CC---CCCCCCCC---EEEEECCEEEEE---CCCC---CCCEE : 281
IPD050Fk : CC---CCCCCCCC---CCEEEECCEEE---EECC---CHHHH : 277
IPD050Fe : CC---CCCCCEEEE---CCCCCCCC---CCCC---CCCC : 229
IPD050F1 : CC---CCCCCEEE---CEEECCCCCCCCCCCC---CCCC : 230
IPD050Fn : CE---ECCCCCCCC---EEEEECCEEE---EECC---CCCC : 232
IPD050Ft : CCCCCCCCCCCCC---EEEEEECCCEEECCCCCCCCCCCCCCCC : 261
IPD050Fh : CCCCEEEECCEEE---EECCCC---CCCCCHHHHH : 279
IPD050Fg : CCCEEEEEECCCEEE---EEEECCCCCEEECCCCCCCCCCCCCCCC : 282
IPD050Fm : CCCEEEEEECCCEEE---CEEEECCEEECCCCCCCCCCCCCHHH : 280
IPD050Fd : CCCEEEEEECCCEEE---EEECCEEECCCCCEEE---CCCCCHHHH : 274
IPD050Fr : CCCEEEEEECCCEEE---CCCCCCCCCCCCCCCC---CCCCCHHHH : 274
IPD050Bb : CCCCCCCCCCCCC--- : 196
IPD050Bd : HHHHCCCCCCCC--- : 196
IPD050Aa : CEEECCEEECCCEEE--- : 197
IPD050Ba : CCCCCCCCCCCCCCH--- : 197
IPD050Bc : CEECCCCCCCCCCCC--- : 196
IPD050Fa : CCCCCCEEEECCEEE---CHHHHHHCCCCCCCCCCCCCCCCCCCC : 248
IPD050Ff : CCCCHHHHHHHHCCCCCHHCCCCCCCCCCCCCCCCCCCCCCCC : 257
IPD050Fq : CCCCHHHHHHHHCCCCCEEEECCEEECCCCCCCCCCCCCCCCCCCC : 250
IPD050Fi : CCCCCCEEEEEEC---CEEEEEECCEEECCCCCCCC---CCCCCC : 229
IPD050Fj : CCCCCCCCCCCCCCCCCCEEEEEECCEEEECCEEEECCEEE---CCCEEE : 254
IPD050Fp : CCCCCCCCCCCCCCEEE---EECCCCCEEECCCC---CEEC : 217
IPD050Gd : CCCCCEEECCEEE--- : 259
IPD050Ga : CCCCCEEECCEEE--- : 257
IPD050Gb : CCCCCCHHCCCCCCCC--- : 257
IPD050Gc : CCCCCEEECCEEE--- : 257

```


Fig. 16j

```

[----- Motif 13 -----][----- motif 2 -----]
          460          *          480          *          500
IPD050Fb : AVVPQLQYDMYSNLIHQMARAAADSYDQSLKQLKLFIAQ---NEILGEYLL : 365
IPD050Fs : AVVPQLQYDMYSNLIHQVARAAADSYDQSLKQLQLFITQ---NEILGDYLL : 372
IPD050Fo : AVVPQLQYDMYSNLIHQVARAAADSYDQSLKQLQLFIAQ---NEILGEYLL : 415
IPD050Fk : AVVPQLQYDMYSNLIHQMARAAADTYDQSLKQFQLFVAQ---NKILGGYLL : 411
IPD050Fe : LVVPQLQYDVYRNLIHQMARVAESYDQSLKQLQLFVAQ---NKILGGYLL : 363
IPD050Fl : LVVPQLQYDVYRNLIHQMARVAESYDQSLKQLQLFLAQ---NKVLGGYLL : 364
IPD050Fn : LVVPQLQYDVYRNLIHQMARVAESYDQSLKQLQLFLAQ---NKILGGYLL : 366
IPD050Ft : LVVPQLQYDVYSNLIHQNRMAQVAESYDQSLRQFKLFIQD---NKILGSYLL : 397
IPD050Fh : FVVPQLQYDTYSPLIHQNRMAQVAESYDQALRQFRLFIQQTQONKILGAFLL : 418
IPD050Fg : LVVPQLQYDVYSNLIHQNRMAQVAESYDQALRQFRLFIQN---NKILGGFLL : 417
IPD050Fm : LVVPQLQYSAYSNLIHQNRMAQVAESYDQALRQFRLFIQQ---NKILGGFLL : 412
IPD050Fd : MVVPQLQYSAYSNLIHQNRMAQVAESYDQALRQFRLFIQQ---NKILGGFLL : 406
IPD050Fr : LVVPQLQYSAYSNLIHQNRMAQVAENYDQALRQFRLFIQQ---NKILGGFLL : 407
IPD050Bb : LVVPRLQYHMYKNLIHQDRMVQVAQNYDQDFRQLKLFVEQ---NKILGSYLL : 319
IPD050Bd : LVVPGLQYRMYKDLIHQDRMVQVAQSYDQDFKQLKLYVEQ---NKILGSYLL : 319
IPD050Aa : LIVPRLQYHMYKDLIHQDRMVQVAQSYDQDFKQLKLFVEQ---NKILGSYLL : 320
IPD050Ba : LIVPRLQYHMYKDLIHQDRMVQVAQSYDQDFKQMKLYVEQ---NKILGSYLL : 320
IPD050Bc : LIVPRLQYHMYKDLIHQDRMVQVAQSYDQDFKQLKLYVEQ---NKILGSYLL : 319
IPD050Fa : FVVPRLQYHMYGSLIHQNRMVQVAQNYDQDFKQLKLFIAQ---NEILGSYLL : 377
IPD050Ff : FVVPRLQYHMYGSLIHQNRMVQVAQNYDQDFKQLKLFIAQ---NKILGSYLL : 387
IPD050Fq : FVVPRLQYHMYGSLIHQNRMVQVAQNYDQDFKQLKLFIAQ---NEILGSYLL : 384
IPD050Fi : SVVPRLQYHMYSDLIHQDRMVQVAQYDDEFQRLRLFIAQ---NEILGSYLL : 363
IPD050Fj : SVVPRLQYHMYSPLIHQNRMVQVAQVYDQDFKQLKLFIVQ---NQILGSYLL : 396
IPD050Fp : LIVPRLQYHMYGSLIHQNRMVQVAQNYDQDFKQLKLFIAQ---NEILGSYLL : 356
IPD050Gd : LKVPLLVYGEFQEDIDQLLRNAEAYDQEYRQLTRFVQQ---VQIIGSEFL : 390
IPD050Ga : LQVPLLVYGEYQEDIDQLLRNAEAYDQEYRELTRFVQQ---VEIIGNEFL : 388
IPD050Gb : LRVPLLVYGEYQEDIERLLRNAEAYDQEYRELTRFVQL---VEIIGSEFL : 388
IPD050Gc : LRVPLLVYGEYQEDIERLLRNAEAYDQEYRALTRFVQL---VEIIGSEFL : 388
IPD050Fb : CCCCCC#####  : 365
IPD050Fs : C#####  : 372
IPD050Fo : C#####  : 415
IPD050Fk : C#####  : 411
IPD050Fe : CCCCCC#####  : 363
IPD050Fl : C#####  : 364
IPD050Fn : C#####  : 366
IPD050Ft : CCCCCC#####  : 397
IPD050Fh : CCCCCC#####  : 418
IPD050Fg : C#####  : 417
IPD050Fm : C#####  : 412
IPD050Fd : C#####  : 406
IPD050Fr : CCCCCC#####  : 407
IPD050Bb : CCCCCC#####  : 319
IPD050Bd : CCCCCC#####  : 319
IPD050Aa : CCCCCC#####  : 320
IPD050Ba : CCCCCC#####  : 320
IPD050Bc : CCCCCC#####  : 319
IPD050Fa : CCCCCC#####  : 377
IPD050Ff : CCCCCC#####  : 387
IPD050Fq : C#####  : 384
IPD050Fi : CCCCCC#####  : 363
IPD050Fj : CCCCCC#####  : 396
IPD050Fp : C#####  : 356
IPD050Gd : CCCCCC#####  : 390
IPD050Ga : CCCCCC#####  : 388
IPD050Gb : CCCCCC#####  : 388
IPD050Gc : CCCCCC#####  : 388

```

helix 4

Fig. 16k

```

-----] [----- motif 12 -----
          *      520          *      540          *
IPD050Fb : EQNRVFAAKERDMEVFHSELIAQKTELQTVMKIDNLSLQMEAQVEDME : 415
IPD050Fs : EQNRVFAAKERDMEVFHSELIAQKTELLTVMAKIDLLSLQMETQAADME : 422
IPD050Fo : EQNRVFAAKERDMEVFHSELISQKTELRTVAKIDLLSLQMETQVADME : 465
IPD050Fk : EQNRAFAAKERDMEVFHSELIAQKTELQTVMKIDKLSLQMDTQVADME : 461
IPD050Fe : EQNRAFAAKERDMEVFHSELIAQKELELKNMVKMEQLSLQMETQIADMD : 413
IPD050Fl : EQNRAFAAKERDMEVFHSELIAQKELELQNTMFKMEQLSSQMETQIADMD : 414
IPD050Fn : EQNRAFAAKERDMEVFHSELIAQKELELQNTMFKMEQLSSQMETQIADMD : 416
IPD050Ft : QQNKAFAADKERDMDVFHSELISQRKIELDNTILKMDQLSLQMESQREDMD : 447
IPD050Fh : DQNRAFAADKERDMDI FYSELIAQRQIELHNTLQKMEQLSLQMETQSADME : 468
IPD050Fg : EQNRAFAEKEKMDMVFYSELITQRQIELDNTLQKLRSLQMETQQADME : 467
IPD050Fm : EQNRAFAEKEQDMDVFYSELIAQRQIELDNTLQKMKHLGAQMETQTADME : 462
IPD050Fd : EQNRAFAEKEQDMDVFYSELITQRQIELDNTLQKMKHLGTMQMDTQTADME : 456
IPD050Fr : EQNRAFAEREQDMDVFYSELITQRKIELDNTLQTMKHLGAQMETQTAGME : 457
IPD050Bb : EQNKAFAEKEKMDAFHSQIIALRTTELNNTIERMGGELSKQMDQENEAME : 369
IPD050Bd : EQNKAFAEKEKMDAFHSQVIALRTTELNNTIERMDDLKQMEEQNAAME : 369
IPD050Aa : EQNKAFAEKEKMDAFHSQVIDLRTSELESTIERMDDLKQMEEQNAAME : 370
IPD050Ba : EQNKAFAEKEKMDASHSQVIALRTSELQSTIERMDDLKQMEVQSTAME : 370
IPD050Bc : DQNKAFAEKEKMDASHSQLIALRTSELESTIERMSDLKQMEVQSKAMK : 369
IPD050Fa : EQNKAFAAKEKSMSEFHLQVSDLRSELNDAIQKMTGLGEEMEVEKEAMD : 427
IPD050Ff : QQNRAFAEREREMSAFHSQVSMRRELQSAIQTMNLSLQMESESEAMN : 437
IPD050Fq : QQNKAFAEREKEMSAFHSQVSMRRELTTA IETMNQLSLQMETESEAMN : 434
IPD050Fi : EQNKALASREREMSAFHSQVSLRRETELDNCLQRMDQLNVQMERENKAME : 413
IPD050Fj : EQNKAFASRETEMSSFHSQVSLRRETELNNAIDRIDQLSVQMENENEAMD : 446
IPD050Fp : QQNKAFAAREKDMFAHGLVVERKQELSSAQETMEELNVQLTQOTEAM : 406
IPD050Gd : QLSMSLAQKERQIETQSLLVIRKQSELDQTMRRIDSLMREIERRSFEMA : 440
IPD050Ga : QLSRSLAEKERQIEI FESLVVIRKQSELDQAIRRMNSLMTEIERRSYEMA : 438
IPD050Gb : QLSRSLAQRERDIEAFESLAVIRKQSELDQAIRRMNSLMPEIERRSFEMA : 438
IPD050Gc : QLSKTLAQRERDIEFESLVVIRKQSELDQAIRRMNSLMTEIERRSFEMA : 438
IPD050Fb : ##### : 415
IPD050Fs : ##### : 422
IPD050Fo : ##### : 465
IPD050Fk : ##### : 461
IPD050Fe : ##### : 413
IPD050Fl : ##### : 414
IPD050Fn : ##### : 416
IPD050Ft : ##### : 447
IPD050Fh : ##### : 468
IPD050Fg : ##### : 467
IPD050Fm : ##### : 462
IPD050Fd : ##### : 456
IPD050Fr : ##### : 457
IPD050Bb : ##### : 369
IPD050Bd : ##### : 369
IPD050Aa : ##### : 370
IPD050Ba : ##### : 370
IPD050Bc : ##### : 369
IPD050Fa : ##### : 427
IPD050Ff : ##### : 437
IPD050Fq : ##### : 434
IPD050Fi : ##### : 413
IPD050Fj : ##### : 446
IPD050Fp : ##### : 406
IPD050Gd : ##### : 440
IPD050Ga : ##### : 438
IPD050Gb : ##### : 438
IPD050Gc : ##### : 438

```

helix 4

Fig. 16o

```

----- Motif 1 -----] [-----
          *           720           *           740           *
IPD050Fb : NVAAVGRELMTTTAYMSQLQYDIQVQAMLQGIASKQADRLSSIQAADLSS : 606
IPD050Fs : NVAAVGRELMTTTAYMSQLQYDIQVQAMLQDIASKQADRLSSIQAADLSS : 613
IPD050Fo : NVAAVGRELMTTTAYMSQLQYDIQVQAMLQDIASKQADRLSSTQAADLSS : 656
IPD050Fk : NVAAVGRELMTTTAYMSQLQYDVQVQAMLQDIARKQADRLSSIQAVDLSS : 652
IPD050Fe : NVAAVGRELMTTTAYISQLQYDIKQVQALLQDIANRQADRLSSIQAADLTS : 604
IPD050Fl : NVAAVGRELMTTTAYISQLQYDIKQVQAMLQDIANKQADRLSSIQAADLSS : 605
IPD050Fn : NVAAVGRELMTTTAYISQLQYDIKQVQAMLQDIASRQADRLSSIQAADLSS : 607
IPD050Ft : NVAVLGREMITTATYISELQYDIKQVQMLQEIATSHANRLSSIQATDLN : 638
IPD050Fh : NVAVLGREMITLATYISELQYDIQMDMLQQIARKQADRLSAIQLSIPNT : 659
IPD050Fg : NVAVLQEMTTTAAAYISQLQYDIMMQLQQIARRQADRLSAIQLPDLTN : 658
IPD050Fm : NVAVLGREMTTLLAAYISQLQYDINMQHMLQQIARKQADRLSAIELPDLRN : 653
IPD050Fd : NVAVLGREMTTLLAAYISQLQYDIEMQHMLQQIARKQADRLSAIQLPDLRN : 647
IPD050Fr : NVAVLGREMTTLLAAYISQLQYDIQMHMLQQIARKQVDRLSAIQLPDLN : 649
IPD050Bb : NVAALGREMSTMAAHIAELQYQIQVQEMLREIAQKQADRLSSISPADLTN : 560
IPD050Bd : NVAALGREMSTMAAHIAELQYQIQVQEMLREIAQKQADRLSSIKPADLTN : 560
IPD050Aa : NVAALGREMSTMAAHIAELQYQIQVQEMLREIAKKQADRLSSIKPADLTN : 561
IPD050Ba : NVAALGREMSTTAAHIAELQYQIQVQEMLQQIAKKQADRLSSIKPADLTN : 561
IPD050Bc : NVAALGREMSTTAVHIAELQYQIQVQEMLQEIARKQAERLSSIKPADLTN : 560
IPD050Fa : NVAVLQEMCTTGSFIADLQYQIKVEMLREIAQQAERLEGISSADLSS : 621
IPD050Ff : NVAVLQAMCTTAAAYISELQYQITVEEMLQEIARQADRLVGISSADLSS : 628
IPD050Fq : NVAVLQAMCTTAAAYMSELQYQITVEEMLQEIARQADRLVGISSADLSS : 625
IPD050Fi : NVAVLQEMCTMAAHIAELQYQIKLEELLQEIADRQADRLLLGISSADLSS : 605
IPD050Fj : NVAVLQEMCTTAAHIGELTYQIKVEEMLQEIARQANRLESITPANLSS : 638
IPD050Fp : NVAAVCRAISTTASYIGLQYELFVHSNQLIARRQAERLEAIQPADLTN : 597
IPD050Gd : NLVAVCREICIAANFAAEVQYELFVHARQEMARRQAERLEGMQLATDLS : 637
IPD050Ga : NLVAVCREICIAASFACEVQYELFVHARQEMARRQAERLEGMQIAADLS : 635
IPD050Gb : NLVAVCREICIAASFACEVQYELFVHARQEMARRQAERLEGMQTAADLS : 635
IPD050Gc : NLVAVCREICIAASFACEVQYELFVHARQEMARRQAERLEGMQTAADLS : 635
IPD050Fb : #####CCCC# : 606
IPD050Fs : #####CCCC# : 613
IPD050Fo : #####CCCC# : 656
IPD050Fk : #####CCCC : 652
IPD050Fe : #####CCCC : 604
IPD050Fl : #####CCCC : 605
IPD050Fn : #####CCCC : 607
IPD050Ft : #####CCCC : 638
IPD050Fh : #####CCCC : 659
IPD050Fg : #####CCCC# : 658
IPD050Fm : #####CCCC : 653
IPD050Fd : #####CCCC : 647
IPD050Fr : #####CCCC : 649
IPD050Bb : #####CCCC : 560
IPD050Bd : #####CCCC# : 560
IPD050Aa : #####CCCC : 561
IPD050Ba : #####CCCC : 561
IPD050Bc : #####CCCC# : 560
IPD050Fa : #####CCCC : 621
IPD050Ff : #####C# : 628
IPD050Fq : #####CCCC# : 625
IPD050Fi : #####CCCC : 605
IPD050Fj : #####CCCC : 638
IPD050Fp : #####CCCC : 597
IPD050Gd : #####CCCC : 637
IPD050Ga : #####C# : 635
IPD050Gb : #####CCCC : 635
IPD050Gc : #####CCCC : 635

```

helix 7

Fig. 16p

```

----- Motif 4 -----] [---
              760          *          780          *          800
IPD050Fb : -FTEMVTEMDMRTTRLLVELIKVLHMQSVALMYQSLTLP-ELMNAWPFVTM : 654
IPD050Fs : -FTEMVTQMDMRTTRLLVELIKVLNMQONVALMYQSLTMP-EPMNGWPFVTM : 661
IPD050Fo : -FTEMVTQMDMRTIRLQVELIKVLDVQONVALMYQSLVKP-ELMNAWPFVTM : 704
IPD050Fk : -FTEMVTQMDMRTTRLLVELIKVLDMQONVALMYQSLIMP-EPINAWPFVTM : 700
IPD050Fe : -YTEMVTQMDMRTTRLLMELIKVLDMQONVALMYQFLTPP-TPMNAWPFVTM : 652
IPD050F1 : -YTEMVTQMDMRTTRLLMELIKVLDMQNAALMYQFLTLP-APMNAWPFVTM : 653
IPD050Fn : -YTEMVTQMDMRTTRLLMELIKVLDMQNAALMYQFLSLP-APMNAWPFVTM : 655
IPD050Ft : -YTEMVTQMDMRTTRMLVALINVVHMQNAALMYQCLSPP-TYVNAWPFVTM : 686
IPD050Fh : GYTEMLMQMDMRTTRLLVALIRAVHIQNAALMYQYLSEP-IPVSAWPFVNM : 708
IPD050Fg : -FTEIVIQMDMRTTRILVALIKVMIQNAALMYQYLSEP-TPVSAWPFVNM : 706
IPD050Fm : -YGELVTQMDMRTTRLLVALIKVNIQNAALMYQYLSEP-TPVYAWPFVNM : 701
IPD050Fd : -YAEVLTQMDMRTTRLLVALIKVNIQNAALMYQYLSEP-TPVYAWPFVNM : 695
IPD050Fr : -YAEVLTQMDMRTTRLLVALIKVNIQNAALMYQYLSEP-TPVYAWPFVSM : 697
IPD050Bb : -YLEMVSQMDMRTTRMLLELIRVLYIQNAALQYEYLQTP-APLNAWPFVAM : 608
IPD050Bd : -YLEMVSQMDMRTTRMLLELIRVLYIQNAALQYEYLQTP-ARLNAWPFVAM : 608
IPD050Aa : -YLEMVSQMDMRTTRMLLELIRVLYIQNAALQYEYLQTP-APLNAWPFVAM : 609
IPD050Ba : -YFEMVSEMDMRTTRMLLELIQVNLIQNGALRYEYLQPA-APLNAWPFVAM : 609
IPD050Bc : -YLEMVSQMDMRTTRMLVELIRVNLIQNGALRYEYLQPA-APLNAWPFVAM : 608
IPD050Fa : -YTEMVSQIDMRTTRLLFQLIKVLHIQNAALKYEYLYAADEHLVSWPFVSM : 670
IPD050Ff : -YTEMASQIDMRTTRILLELIKMLYIQNAAIKYEYLYDANEKLNWSWPFVSM : 677
IPD050Fq : -YTEMASQIDMRTTRILLELIKMLYIQNAAIKYEYLYDANEKLNWSWPFVSM : 674
IPD050Fi : -FTEMLTQIDMRTTRLLQLIKLLHIQNVAINYEYLFPPANGRLSSWPFVSM : 654
IPD050Fj : -YTEMLSEIDMRTTRLLQLIRLLHIQNAAIKYEYLYAAGEQLNWSWPFVSM : 687
IPD050Fp : -SLEMATQIDMRTSRMLLNLLKVLTLQSGALQFHFLPP-TPFTGW-VNM : 644
IPD050Gd : SYIELATQADMRTTRLLSLLNVLADHQGALHYHYLMELQVFTFTWPSVD : 687
IPD050Ga : SYIELATQADMRTTRLLSLLNVLADHQGALHYHYLMELEVFTFSWPSVD : 685
IPD050Gb : SYIELATQADMRTARLLSLLNVLADHQGALHYHYLMELEVFTSSWPTVD : 685
IPD050Gc : SFIELATQADMRTTRLLSLLNVLADHQGALHYHYLMELEVFTSSWPSVD : 685
IPD050Fb : -#####CCCCC-CCCCCCCC# : 654
IPD050Fs : -#####CCCCC-CCCCCCCC# : 661
IPD050Fo : -#####CCCCC-CCCCCCCC# : 704
IPD050Fk : -#####CCCCC-CCCCCCCC# : 700
IPD050Fe : -#####CCCCC-CCCCCCCC# : 652
IPD050F1 : -#####CCCCC-CCCCCCCC# : 653
IPD050Fn : -#####CCCCC-CCCCCCCC# : 655
IPD050Ft : -#####CCCCC-CCCCCCCC# : 686
IPD050Fh : C#####CCCCC-CCCCCCCC# : 708
IPD050Fg : -#####CCCCC-CCCCCCCC# : 706
IPD050Fm : -#####CCCCC-CCCCCCCC# : 701
IPD050Fd : -#####CCCCC-CCCCCCCC# : 695
IPD050Fr : -#####CCCCC-CCCCCCCC# : 697
IPD050Bb : -#####CCCC-CCCCC### : 608
IPD050Bd : -#####CCCC-CCCCCCCC# : 608
IPD050Aa : -#####CCCC-CCCCCCCC# : 609
IPD050Ba : -#####CCCC-CCCCCCCC# : 609
IPD050Bc : -#####CCCCC-CCCCCCCC# : 608
IPD050Fa : -#####CCCCCCCCCCCC# : 670
IPD050Ff : -#####CCCCCCCCCCCC# : 677
IPD050Fq : -#####CCCCCCCCCCCC# : 674
IPD050Fi : -#####CCCCCCCCCCCC# : 654
IPD050Fj : -#####CCCCCCCCCCCC# : 687
IPD050Fp : -#####CCCCC-CCCCC-C# : 644
IPD050Gd : #####CCCCCCCCCCCC# : 687
IPD050Ga : #####CCCCCCCCCCCC# : 685
IPD050Gb : #####CCCCCCCCCCCC# : 685
IPD050Gc : #####CCCCCCCCCCCC# : 685

```

helix 8

Fig. 16r

Motif 22 -][----- motif 10 -----] [---
860 * 880 * 900

IPD050Fb : WEFEISVDD-FTVFPRTWSRVRIHHLEMKFVGSDEAAPGGGMQGAHQPA : 749
IPD050Fs : WEFEISVDD-FTAFPATWSRVRIHHLEMKFVGS-EEAAPWG-MQGP HQPA : 754
IPD050Fo : WEFEIPVDD-FRVFPATWSRVRIHHLEMKFAAA-----PAG-TQGAHQPA : 793
IPD050Fk : WEFEIPVDD-FTVFPVTWSRVRIHHLEMKFVGT-DEAAPAPGLQGT HQPT : 794
IPD050Fe : WQFEIPVNE-RTVFPSTWSQVRIHHLEMKFVGSNN-IDHEG----SHQPI : 742
IPD050Fl : WQFEIPVNE-LTVFPSTWSQVRIHHLEMKFVGTAEGSAPNG----AHLPI : 744
IPD050Fn : WQFEIPVNE-LTVFPSTWSQVRIHHLEMKFVGTAEGSAPNG----AHQPI : 746
IPD050Ft : WEFEISVED-YTTFPSTWSRVRIHHLEMKFVQAAD-----VHQPI : 771
IPD050Fh : YEFDIPVED-NITFPLSLSRVRIHHLEMKFVAS-----GGQ-KQVHMPV : 796
IPD050Fg : YQFEIPVED-PLTFPLSLSRVRIHHLEMKIVQG-----AHVHTPI : 791
IPD050Fm : YEFEIPAQD-HVTFFPLSLSRVRIHHLEMKFVQ-----GEG--DQLHMPV : 788
IPD050Fd : YEFEIPVEN-HITFPLSLSRVRIHHLEMRVQ--DGDTAQG--AHVHMPI : 786
IPD050Fr : YEFELPVED-HITFPLSLSRVRIHHLEMKFVQGAAGGSDQGT-QIVHMPI : 791
IPD050Bb : WEFELPVLN--ADFPSTWSRVRIHHVDMQFD-----AAATSIHPT : 693
IPD050Bd : WEFELPMN--ADFPSTWSRVRIHHVDMQFD-----AGAK--HLPS : 691
IPD050Aa : WEFELPVRN--ADFPSTWSRVRIHYVDMRFD-----AAAE--HLPV : 692
IPD050Ba : WEFELPVD--ADFPSTWSRVRIHYVDMQFD-----AAAE--HLPT : 692
IPD050Bc : WEFELPVD--DDFPSTWSRVRIHYVDMQFD-----AAAE--HLPT : 691
IPD050Fa : WPFVIHEED-YTAFP SGWYVRIHVELKFEQQ----TAGSTDIVHQPS : 765
IPD050Ff : WNFEIAVED-FAIFPSGLSRVRIHYVELKFDQQ----GADSSNIVHQPS : 768
IPD050Fq : WNFEIAVED-FSIFPSGWSRVRIHYVELKFDQQ----GTDSSNIVHQPS : 765
IPD050Fi : WQFAIPVMDSSAFP IGF SRVRIHVELKFSAI----DIDAS-ATVHQPR : 745
IPD050Fj : WQFSIPVED-SPTFPIGYSRVRIHYVELRFDQG---TSDEG-RMIVHQPS : 777
IPD050Fp : WIFTINPLD-GSTFPLSWSRVRIHYLEMKFTGG-----EEFHKPT : 729
IPD050Gd : WSFTINPGQNASVFPAPWDYVRIHYVEMKFTG-----EHPV : 773
IPD050Ga : YNFTINPERNASSFPPTWDYVRIHYVEVKFTG-----EHQPV : 771
IPD050Gb : WNFTINPERNASSFPPTWDYVRIHYVEMRFTG-----EHLPV : 771
IPD050Gc : WSFTINPERNASAFPPTWDYVRIHYVEMKFTG-----EHLPV : 771
IPD050Fb : CCC-CCCCCCCCC-----CCCCCCCCCCCCCCCC : 749
IPD050Fs : CCC-CCCCCCCCC-----CCCCCCCC-----CCCCCCC : 754
IPD050Fo : CCC-CCCCCCCCC-----CCC-CCCCCCCC : 793
IPD050Fk : CCC-CCCCCCCCC-----CCCCCCCCCCCCCCCC : 794
IPD050Fe : CCC-CCCCCCCCC-----CCC-CCCC-----CCCC : 742
IPD050Fl : CCC-CCCCCCCCC-----CCCCCCCC-----CC : 744
IPD050Fn : CCC-CCCCCCCCC-----CCCCCCCC-----CCCC : 746
IPD050Ft : CCC-----CCCC : 771
IPD050Fh : CCC-CCCCCCCC-----CCC-CCCCCCCC : 796
IPD050Fg : CCC-CCCCCCCC-----CCCCCCCC : 791
IPD050Fm : CCC-CCCCCCCC-----CCC-CCCCCCCC : 788
IPD050Fd : CCC-CCCCCCCC-----CCCCCCCC-----CC : 786
IPD050Fr : CCC-CCCCCCCC-----CCCCCCCC-----CC : 791
IPD050Bb : CCC-CCCCCCCC-----CCCCCCCC : 693
IPD050Bd : CCC-CCCCCCCC-----CCCC-CCCC : 691
IPD050Aa : CCC-CCCCCCCC-----CCCC-CCCC : 692
IPD050Ba : CCC-CCCCCCCC-----CCCC-CCCC : 692
IPD050Bc : CCC-CCCCCCCC-----CCCC-CCCC : 691
IPD050Fa : CCC-CCCCCCCC-----CCCCCCCCCCCC : 765
IPD050Ff : CCC-CCCCCCCC-----CCCCCCCCCCCC : 768
IPD050Fq : CCC-CCCCCCCC-----CCCCCCCCCCCC : 765
IPD050Fi : CCC-CCCCCCCC-----CCCC-CCCCCCCC : 745
IPD050Fj : CCC-CCCCCCCC-----CCCC-CC : 777
IPD050Fp : CCC-CCCCCCCC-----CCCCCCCC : 729
IPD050Gd : CCC-CCCCCCCC-----CCCC : 773
IPD050Ga : CCC-CCCCCCCC-----CCCC : 771
IPD050Gb : CCC-CCCCCCCC-----CCCC : 771
IPD050Gc : CCC-CCCCCCCC-----CCCC : 771

β1

β2

Fig. 16s

- Motif 17 ---] [----- motif 3 -----

* 920 * 940 *

IPD050Fb : TKTGEVYILLQSSRVFHDRNKT--KPLHYEAGIPLDYHYAYNLETGETTL : 797

IPD050Fs : TKSGEIYILLQSSRVFHDRNKT--KPLHYEAGIPLDYHYAYNLTGETTL : 802

IPD050Fo : TKSGEIYILLQSSRVFHDRNKA--EPLHYEAGVPLDYHYAYNLETGETTL : 841

IPD050Fk : TKTGEIYILLQSSRVFHDRNNT--KPLHYEAGVPLDYHYAYNLETGETTL : 842

IPD050Fe : TESGEVYILLQSSRVFHDRNKS--ELLHYEAAVPLDYHYAYHLETGETTL : 790

IPD050Fl : TESGEVYILLQSSRVFHDRNKT--EPLHYEAAVPLDYHYAYHLKTGETTL : 792

IPD050Fn : TESGEVYILLQSSRVFHDRNKT--EPLHYEAAVPLDYHYAYHLKTGETTL : 794

IPD050Ft : TDTGKVMYLLQSSRFVHDRNRA--DAIRYEAAVSLDYHYAYRLDTGETTL : 819

IPD050Fh : TDSGSVYILLQSSRFVHDRNER--AVVHYEAAVSLDYHYAYNLETGQIKV : 844

IPD050Fg : TATGKIYILLQSSRFVHDRNEG--KIMHYEAAVPLDYHYAYQLDTGETTV : 839

IPD050Fm : TDTGSVYILLQSSRFVHDRSEG--TILHYEAAVSLDYHFAYRLDTGETTV : 836

IPD050Fd : TDTGSIYILLQSSRFVHDRNEG--AILHYEAAVPLDYHFAYRLDTGETTV : 834

IPD050Fr : TDTGSIYILLQSSRFVHDRNES--AILHYEAAVSLDYHFAYRLDTGASTV : 839

IPD050Bb : TNTGVVYLLQSSRFVDDRARRANEFSYEAGTGLFYQYAYRLATGEATV : 743

IPD050Bd : TSTGQVYLLQSSRFVADRKRANEYINYQAGTGLYYQYAYRLATGEATV : 741

IPD050Aa : TSTGEVYLLQSSRFVDRKRANEFSYEGGMGLQYQYAYRLATGDATV : 742

IPD050Ba : TSTGEVYLLQSSRFVDRKQHEDEFISYEAGTGLQYQYAYRLATGEATV : 742

IPD050Bc : TSTGEVYLLQSSRFVDRKQHEDEFVSYEAGTGLQYQYAYRLDTGEATV : 741

IPD050Fa : TNTGRLYMLLQSSRFVHDRNQG--VKMEYEGSRGLSYPYAYNLTGETTH : 813

IPD050Ff : TNTGLVYMLLQSSRFVHDRKRQ--EVMDYEAAVPLDYHYAYDLNTGATTL : 816

IPD050Fq : TNTGLVYMLLQSSRFVHDRKRQ--EVMDYEAAVPLDYHYAYDLNTGATTL : 813

IPD050Fi : TDNGFIYILLQSSRFVDRRVR--QAMDYEAAVPLDYHYAYNLTGVPSTL : 793

IPD050Fj : TSSGLTYMLLQSSRFVDRRVR--EVLEYEAAVPLDYHYAYNLTGVPSTL : 825

IPD050Fp : TDSGKVMYLLQSSRFVDRRVR--EVLHYEAAVPLDYHYAYNLTGGETTV : 777

IPD050Gd : SQTGEVYLLQSSRFVDRRVR--QVLEYEAAVPLDYHYAYNLTGATTL : 821

IPD050Ga : TKTGEIYLLRSSANFQDRLEE--QVFEYEAAVPLDYHYAYNLTGATTL : 819

IPD050Gb : SETGEIYLLRSSANFQDRLEE--QVFEYEAAVPLDYHYAYNLTGATTL : 819

IPD050Gc : TDTGEIYLLRSSANFQDRLEE--QVFEYEAAVPLDYHYAYNLTGATTL : 819

IPD050Fb : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 797

IPD050Fs : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 802

IPD050Fo : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 841

IPD050Fk : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 842

IPD050Fe : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 790

IPD050Fl : ECCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 792

IPD050Fn : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 794

IPD050Ft : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 819

IPD050Fh : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 844

IPD050Fg : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 839

IPD050Fm : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 836

IPD050Fd : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 834

IPD050Fr : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 839

IPD050Bb : CCCC[REPEAT]CCCCCCCCCCCCCCCCCCCCCCCC[REPEAT]CCCCCCCCCCCC : 743

IPD050Bd : CCCC[REPEAT]CCCCCCCCCCCCCCCCCCCCCCCC[REPEAT]CCCCCCCCCCCC : 741

IPD050Aa : CCCC[REPEAT]CCCCCCCCCCCCCCCCCCCCCCCC[REPEAT]CCCCCCCCCCCC : 742

IPD050Ba : CCCC[REPEAT]CCCCCCCCCCCCCCCCCCCCCCCC[REPEAT]CCCCCCCCCCCC : 742

IPD050Bc : CCCC[REPEAT]CCCCCCCCCCCCCCCCCCCCCCCC[REPEAT]CCCCCCCCCCCC : 741

IPD050Fa : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 813

IPD050Ff : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 816

IPD050Fq : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 813

IPD050Fi : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 793

IPD050Fj : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 825

IPD050Fp : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 777

IPD050Gd : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 821

IPD050Ga : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 819

IPD050Gb : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 819

IPD050Gc : CCCC[REPEAT]CCCCCCCCCCCC--CC[REPEAT]CCCC[REPEAT]CCCCCCCC : 819

β3 β4 β5

Fig. 16u

```

-- Motif 14 ----]
          *      1020      *
IPD050Fb : TTQIAITFHVSAIREIAL----- : 861
IPD050Fs : TTQIAITFHVSAIREIAF----- : 866
IPD050Fo : TTQIAITFHVSAIREIAL----- : 905
IPD050Fk : TTQIAVTFHVSAREIAL----- : 906
IPD050Fe : TTQIAVTFSVSAIREISL----- : 854
IPD050F1 : TTQIAVTFYVSAIREISL----- : 856
IPD050Fn : TTQIAVTFYVSAIREISL----- : 858
IPD050Ft : TTQIAIIFYISAIRRISL----- : 882
IPD050Fh : TTQIVVTFHISAVRQVAF----- : 907
IPD050Fg : TTEIAITFHISAIRRISD----- : 902
IPD050Fm : TTHIAITFFISAIRQIAL----- : 899
IPD050Fd : TTQIAITFFISAIRQIAL----- : 897
IPD050Fr : TTHIAITFFISAIRQVAL----- : 903
IPD050Bb : TTQIKITFHVSARRISTRSDGVSS----- : 813
IPD050Bd : TTQIKITFHVSARRISTFVADPK----- : 810
IPD050Aa : TTRIKITFHVSARRISTRVAV----- : 809
IPD050Ba : TTRIKITFHVSARRISLAR----- : 807
IPD050Bc : TTHIKITFHVSARRISLSR----- : 806
IPD050Fa : TTQVSITFHVTRIRRIDRFTSTVHQKHAS----- : 888
IPD050Ff : TTQISITFYVTAIRRIDLRQEGDVE----- : 886
IPD050Fq : TTQISITFYVTAIRRIDHRQEGDEE----- : 884
IPD050Fi : STQISITFHITAIRAIDFRGMDDEERSKSDSSQK : 872
IPD050Fj : TTQITITFFISAIRGIDTRAAVEI----- : 895
IPD050Fp : TTQISITFFVTAVRAVDFRSLDDQDM----- : 851
IPD050Gd : SIQITITFYVTALPQIQSLS-EDPAVATTL---- : 893
IPD050Ga : PVQITITFYVTALPQIQTSS-TDQFASTT---- : 895
IPD050Gb : PVQITITFFVTALPQIQSRS-LDMSDGDPTEE-- : 896
IPD050Gc : PVRITITFYVTALPQIQSRSLDMST----- : 892
IPD050Fb : CCCCCCCCCCHHHHHCCC----- : 861
IPD050Fs : CCCCCCCCCCHHHHHHCC----- : 866
IPD050Fo : CCCCCCCCCCHHHHHHCC----- : 905
IPD050Fk : CCCCCCCCCCHHHHHHHC----- : 906
IPD050Fe : CCCCCCCCCCCHHHCCC----- : 854
IPD050F1 : CCCCCCCCCCHHHHCCC----- : 856
IPD050Fn : CCCCCCCCCCHHHHCCC----- : 858
IPD050Ft : CCCCCCCCCCCHCCC----- : 882
IPD050Fh : CCCCCCCCCCHHHCCC----- : 907
IPD050Fg : CCCCCCCCCCCHCCC----- : 902
IPD050Fm : CCCCCCCCCCHHHHHHHC----- : 899
IPD050Fd : CCCCCCCCCCHHHHHHHC----- : 897
IPD050Fr : CCCCCCCCCCHHHHHHHC----- : 903
IPD050Bb : CCCCCCCCCCCHCCCCCCCC----- : 813
IPD050Bd : CCCCCCCCCCCHCCCCCCCC----- : 810
IPD050Aa : CCCCCCCCCCCHCCCC----- : 809
IPD050Ba : CCCCCCCCCCCHCCCC----- : 807
IPD050Bc : CCCCCCCCCCCHCCCC----- : 806
IPD050Fa : CCCCCCCCCCCHHHHHHCCCC----- : 888
IPD050Ff : CCCCCCCCCCCHHHHHCCCCCCCC----- : 886
IPD050Fq : CCCCCCCCCCCHHHHHCCCCCCCC----- : 884
IPD050Fi : CCCCCCCCCCCHHHHHCCCCCCCCCCCCCCCC : 872
IPD050Fj : CCCCCCCCCCCHHHHHCCCCCCCC----- : 895
IPD050Fp : CCCCCCCCCCCHHHHHCCCCCCCC----- : 851
IPD050Gd : CCCCCCCCCCCHHHHHCCCCCCCC----- : 893
IPD050Ga : CCCCCCCCCCCHHHHHCCCCCCCC----- : 895
IPD050Gb : CCCCCCCCCCCHHHHHCCCCCCCC----- : 896
IPD050Gc : CCCCCCCCCCCHHHHHCCCCCCCC----- : 892

```

β7

Fig. 17a

	*	20	*	40	*	
PtIP-65Gc	:	-----	:	-----	:	-
PtIP-65Ha	:	-----	:	-----	:	-
PtIP-65Gd	:	-----	:	-----	:	-
PtIP-65Ge	:	-----	:	-----	:	-
PtIP-65Ga	:	-----	:	-----	:	-
PtIP-65Gb	:	-----	:	-----	:	-
PtIP-65Hj	:	-----	:	-----	:	-
PtIP-65Hk	:	-----	:	-----	:	-
PtIP-65Hg	:	MQYGLANTEASPLIEKFQALMEGGIDESILATKLVGAEGDASHLPPPGET	:		:	50
PtIP-65Hh	:	-----MAQLQQHVVN-----	:		:	10
PtIP-65Fa	:	-----	:	-----	:	-
PtIP-65Fb	:	-----	:	-----	:	-
PtIP-65Ca	:	-----	:	-----	:	-
PtIP-65Aa	:	-----	:	-----	:	-
PtIP-65Ba	:	-----	:	-----	:	-
PtIP-65Bb	:	-----	:	-----	:	-
PtIP-65He	:	-----	:	-----	:	-
PtIP-65Hf	:	-----	:	-----	:	-
PtIP-65Hb	:	-----	:	-----	:	-
PtIP-65Hc	:	-----	:	-----	:	-
PtIP-65Hd	:	-----	:	-----	:	-
PtIP-65Gc	:	-----	:	-----	:	-
PtIP-65Ha	:	-----	:	-----	:	-
PtIP-65Gd	:	-----	:	-----	:	-
PtIP-65Ge	:	-----	:	-----	:	-
PtIP-65Ga	:	-----	:	-----	:	-
PtIP-65Gb	:	-----	:	-----	:	-
PtIP-65Hj	:	-----	:	-----	:	-
PtIP-65Hk	:	-----	:	-----	:	-
PtIP-65Hg	:	CCCCCCCCCCHHHHHHHHHHHCCCCHHEEEEEEECCCCCCCCCCCCCCC	:		:	50
PtIP-65Hh	:	-----CCCCCEEEEE-----	:		:	10
PtIP-65Fa	:	-----	:	-----	:	-
PtIP-65Fb	:	-----	:	-----	:	-
PtIP-65Ca	:	-----	:	-----	:	-
PtIP-65Aa	:	-----	:	-----	:	-
PtIP-65Ba	:	-----	:	-----	:	-
PtIP-65Bb	:	-----	:	-----	:	-
PtIP-65He	:	-----	:	-----	:	-
PtIP-65Hf	:	-----	:	-----	:	-
PtIP-65Hb	:	-----	:	-----	:	-
PtIP-65Hc	:	-----	:	-----	:	-
PtIP-65Hd	:	-----	:	-----	:	-

Fig. 17b

	60	*	80	* motif 12	100			
PtIP-65Gc	-----MALYQTPVYVIGGQ-GGS-					17		
PtIP-65Ha	-----MALYQTPVYVIGGQ-GGS-					17		
PtIP-65Gd	-----MALYQTPVYVIGGQ-GGN-					17		
PtIP-65Ge	-----MALYQTPVYVIGGQ-GGN-					17		
PtIP-65Ga	-----MSLVQTPVYVIGGQ-GGN-					17		
PtIP-65Gb	-----MSLVQTPVYVIGGQ-GGN-					17		
PtIP-65Hj	-----MSIHQTPVTLIGGR-GGA-					17		
PtIP-65Hk	-----MSSGSVIGGVTMVGGP-YGS-					19		
PtIP-65Hg	PSEDGAGKDPPN-ESLETEDVEEHADDSKARSASVTAPLRFIGGP-GGS-					97		
PtIP-65Hh	-SKHAYGKHAPASKVCEIARAPVHAYKG-SNQGDTVAPLTFIGGD-GGK-					56		
PtIP-65Fa	-----MAFQTPVTLIGASSGGQ-					17		
PtIP-65Fb	-----MAFQTPVTLIGSSGGQ-					17		
PtIP-65Ca	-----MALVIG--GLIGGG-GGS-					15		
PtIP-65Aa	-----MALVIG--GLIGGQ-GGY-					15		
PtIP-65Ba	-----MALVIS--CPVGGQ-GGS-					15		
PtIP-65Bb	-----MALVIG--WPVGGQ-GGS-					15		
PtIP-65He	-----MQPVQLAGSVNTSLG					15		
PtIP-65Hf	-----MTSLLTPPYMQPIQSAGGLFSSF-					23		
PtIP-65Hb	-----MSLLT-PHLLTASAGG-FIGGD-					20		
PtIP-65Hc	-----MSSLIN-PHLETIRYGGSFAGGS-					22		
PtIP-65Hd	-----MSSLIN-PHLESITYAGSFAGGS-					22		
PtIP-65Gc	-----CC			EEEEEEEE	CCC-CCC-	17		
PtIP-65Ha	-----CC			EEEEEEEE	CCC-CCC-	17		
PtIP-65Gd	-----CC			EECCC	EEEEE	CCC-CCC-	17	
PtIP-65Ge	-----CC			EECCC	EEEEE	CCC-CCC-	17	
PtIP-65Ga	-----CCCCC			EEEEEEEE	CC-CCC-	17		
PtIP-65Gb	-----CCCCC			EEEEEEEE	CCC-CCC-	17		
PtIP-65Hj	-----CC			EEEEEEEE	CCCC-CCC-	17		
PtIP-65Hk	-----CCCCCCCCC			EEEEE	CCC-CCC-	19		
PtIP-65Hg	CCCCCCCCCCCC-CCCCCCCCCCCCCCCCCCCCCCCC			EEEEE	CCC-CCC-	97		
PtIP-65Hh	-CCCCCCCCCCCC			EEEEE	CC	EEEEE	CCC-CCC-	56
PtIP-65Fa	-----CCCCC			EEEEE	CCCCCCC-	17		
PtIP-65Fb	-----CCCCC			EEEEE	CCCCCCC-	17		
PtIP-65Ca	-----CC			EEEC	CCCCC-CCC-	15		
PtIP-65Aa	-----CC			EEEC	CCCCC-CCC-	15		
PtIP-65Ba	-----CC			EEEC	CCCCC-CCC-	15		
PtIP-65Bb	-----CC			EEEC	CCCCC-CCC-	15		
PtIP-65He	-----CCC			EEEEE	CCCCCCCC	15		
PtIP-65Hf	-----CCCCCCCC			EEEEE	CCCCCCCC	23		
PtIP-65Hb	-----CCCCC			EEEEE	CC-CCCC-	20		
PtIP-65Hc	-----CCCCC			EEEEE	CCCCCCC-	22		
PtIP-65Hd	-----CCCCC			EEEEE	CCCCCCC-	22		

β1

Fig 17e

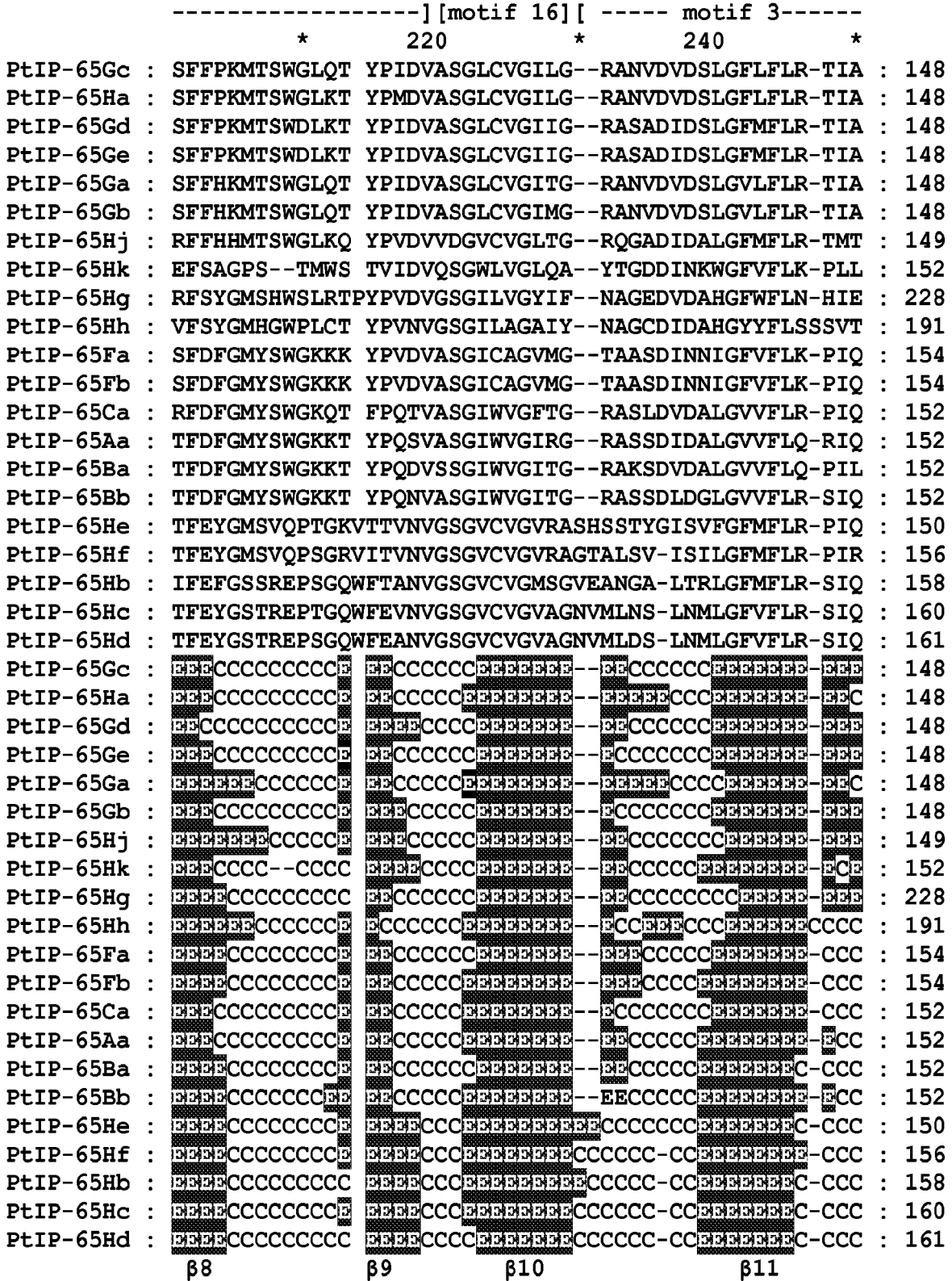


Fig 17f

```

-----] [--- motif 15 ---] [--- motif 7
          260          *          280          *          300
PtIP-65Gc : SARMINVSYPTLGLEQAG-----IIPITLDSYNDSSNAGAI SKN----- : 187
PtIP-65Ha : SARMINVSYPTLGLEQAG-----IIPITLDSYNDSSNAGAI SKN----- : 187
PtIP-65Gd : SSRMINVSYPTLGLEQAG-----IIPVTLDSYNDSSNAGSISK N----- : 187
PtIP-65Ge : SSRMINVSYPTLGLEQAG-----IIPVTLDSYNDSSNAGSISK N----- : 187
PtIP-65Ga : PARMINVSYPTLGLEQAG-----IIPVTLDSYNDSSNAGTISK N----- : 187
PtIP-65Gb : SARMINVSYPTLGLEQAG-----IIPVTLDSFNDSNAGTISK N----- : 187
PtIP-65Hj : SARMINVKYPTLGLTAG-----IVPVTLD FMSDSNASSISK T----- : 188
PtIP-65Hk : VFALADVQYTGL-QDVGA-----IVPTTLD SLDETNNSS TGS DVN----- : 191
PtIP-65Hg : QAELTNVRYPTLGFDTAG-----IVPTALD TFRFRNNS ST--PRD----- : 266
PtIP-65Hh : SSKLENVKYPTLKFDTS-----ITPVSLD TYKQTNTSSS--PRN----- : 229
PtIP-65Fa : SSKLINVQYPSLSFDTQG-----ISPQTLKEFNHTN-TSNNPTN----- : 192
PtIP-65Fb : SSKLINVQYPSLSFDTQG-----ISPQTLKEFNHTN-TSNNPTS----- : 192
PtIP-65Ca : SCQLMNVQYPTLQFSGSSGATSITPTASSTKSFTLLNTADHEDQSST--- : 199
PtIP-65Aa : SCRLTSVQYPTLQFSGSSGGTTSIVRTPTKSFNLGNTADQDDPSST--- : 199
PtIP-65Ba : SCRLISVDYPTLQFSGTSGGTTSINLTTAGKTFNLQNAAHQEDPSST--- : 199
PtIP-65Bb : SCRLINVQYPTLQFSGTAGGTTSITRTTAAKTFNLQNTANQDDPSST--- : 199
PtIP-65He : SVRLHGLVYPTISSSTITTTTTILQELPATIKNDNDHEPLHWVLAGSRQCI : 200
PtIP-65Hf : SVRLHGLVYPTISSISTITTTTTILQELPATLKNDDHEPLHWVLAGSRQCF : 206
PtIP-65Hb : SVGFSSVEYPTLSTSTILTPILEQLPDTFKSNDDDEPLHVLAGSRQLE : 208
PtIP-65Hc : SVGFSSVEYPMFSTS-ITRTSILEQLPDTFKSNDDDEPLQMVLAGSRQFK : 209
PtIP-65Hd : RVGFSSVEYPTISSS-IARTFILSHLPDTFKSNDDDEPLQMVLAGSRQFK : 210
PtIP-65Gc : ██████████CCCCCCCC-----CC████████████████████ : 187
PtIP-65Ha : CCC█████████CCCCCCCC-----CCC████████████████████ : 187
PtIP-65Gd : ██████████CCCCCCCC-----CCC████████████████████ : 187
PtIP-65Ge : ██████████CCCCCCCC-----CCC████████████████████ : 187
PtIP-65Ga : CCCCCCCCCCCCCCCCC-----CCC████████████████████ : 187
PtIP-65Gb : ██████████CCCCCCCC-----CCC████████████████████ : 187
PtIP-65Hj : ██████████CCCCCCCC-----CCC████████████████████ : 188
PtIP-65Hk : ██████████CCC-CCCC-----CCC████████████████████ : 191
PtIP-65Hg : ██████████CCCCCCCC-----CC████████████████████ : 266
PtIP-65Hh : C█████████CCCCCCCC-----CCC████████████████████ : 229
PtIP-65Fa : CC█████████CCCCCCCC-----CCC████████████████████ : 192
PtIP-65Fb : ██████████CCCCCCCC-----CCC████████████████████ : 192
PtIP-65Ca : ██████████CCCCCCCCCCCCCCCCCCCC████████████████████ : 199
PtIP-65Aa : ██████████CCCC████████████████████████████████████████ : 199
PtIP-65Ba : C█████████CCCCCCCCCCCC████████████████████████████████ : 199
PtIP-65Bb : ██████████CCCC████████████████████████████████████████ : 199
PtIP-65He : ██████████CCCCCCCC████████████████████████████████████████ : 200
PtIP-65Hf : ██████████C████████████████████████████████████████████ : 206
PtIP-65Hb : ██████████CCCCCCCCCCCC████████████████████████████████ : 208
PtIP-65Hc : ██████████CCCCCCC-CCCC████████████████████████████████ : 209
PtIP-65Hd : ██████████CCCCCCC-CCC████████████████████████████████ : 210

```

β12

Fig 17j

	460	* *	480	* *	500	
PtIP-65Gc :	TNTGTR-----	ALKNEVEVEAVDQ-----	-----	QSQ-EGDHNVPNK----	-----	: 343
PtIP-65Ha :	TNTGTR-----	ALKNEVEVEAVDQ-----	-----	QSQ-EGDHNVPNK----	-----	: 343
PtIP-65Gd :	TNTGTR-----	ALK-QVEVQATDQ-----	-----	QSQ-EGDHNVPDK----	-----	: 342
PtIP-65Ge :	TNTGTR-----	ALK-QVEVQATDQ-----	-----	QSQ-EGDHNVPDK----	-----	: 342
PtIP-65Ga :	TNTGTR-----	ASD-HVEVEATEQ-----	-----	QVQGVKDQSVQPNK----	-----	: 343
PtIP-65Gb :	TNTGTR-----	ASD-HVEVEATEQ-----	-----	QVQGVKDQSVQPNK----	-----	: 343
PtIP-65Hj :	VSQGTRDLGSDHLA	INKDVRYIAAANGAAVGT	TTTTNAPPHYVHPI	IRGAPI		: 362
PtIP-65Hk :	DVKVT-----	-----	-----	-----	-----	: 321
PtIP-65Hg :	NDNST-----	-----	-----	-----	-----	: 395
PtIP-65Hh :	KDHEETKTAMAMKSYASLCL	-----	-----	-----	-----	: 373
PtIP-65Fa :	T-----	-----	-----	-----	-----	: 317
PtIP-65Fb :	T-----	-----	-----	-----	-----	: 317
PtIP-65Ca :	TSQ-----	-----	-----	-----	-----	: 341
PtIP-65Aa :	TTQ-----	-----	-----	-----	-----	: 339
PtIP-65Ba :	TTQ-----	-----	-----	-----	-----	: 340
PtIP-65Bb :	TAQ-----	-----	-----	-----	-----	: 339
PtIP-65He :	LS-----	-----	-----	-----	-----	: 351
PtIP-65Hf :	LS-----	-----	-----	-----	-----	: 356
PtIP-65Hb :	IDP-----	-----	-----	-----	-----	: 355
PtIP-65Hc :	L-----	-----	-----	-----	-----	: 356
PtIP-65Hd :	L-----	-----	-----	-----	-----	: 358
PtIP-65Gc :	EEECCC-----	CCCCCHHHHHHCC-----	-----	EEC-CCCCCCCCC-----	-----	: 343
PtIP-65Ha :	EEECCC-----	CCCCCHHHHHHCC-----	-----	EEC-CCCCCCCCC-----	-----	: 343
PtIP-65Gd :	EEEEEE-----	CCC-CCCCCCCCC-----	-----	CCC-CCCCCCCCC-----	-----	: 342
PtIP-65Ge :	EEEEEE-----	CCC-CCCCCCCCC-----	-----	CCC-CCCCCCCCC-----	-----	: 342
PtIP-65Ga :	EEECCC-----	CCC-CEEEEEEEEC-----	-----	EECCCCCCCCCCCC-----	-----	: 343
PtIP-65Gb :	EEECCC-----	CCC-CCEEHHEEE-----	-----	EECCCCCCCCCCCC-----	-----	: 343
PtIP-65Hj :	EEEECCCCCCCCCCCC	EEEEEECCCCEEEEEE	CCCCCCCCCCCCCCCC	CCCCCCCCCCCCCCCC	CCCCCCCCCCCCCCCC	: 362
PtIP-65Hk :	EEEEEC-----	-----	-----	-----	-----	: 321
PtIP-65Hg :	EECCC-----	-----	-----	-----	-----	: 395
PtIP-65Hh :	EECCCC	EEEECCCCCCCC	-----	-----	-----	: 373
PtIP-65Fa :	C-----	-----	-----	-----	-----	: 317
PtIP-65Fb :	C-----	-----	-----	-----	-----	: 317
PtIP-65Ca :	ECC-----	-----	-----	-----	-----	: 341
PtIP-65Aa :	EEC-----	-----	-----	-----	-----	: 339
PtIP-65Ba :	ECC-----	-----	-----	-----	-----	: 340
PtIP-65Bb :	ECC-----	-----	-----	-----	-----	: 339
PtIP-65He :	EC-----	-----	-----	-----	-----	: 351
PtIP-65Hf :	CC-----	-----	-----	-----	-----	: 356
PtIP-65Hb :	ECC-----	-----	-----	-----	-----	: 355
PtIP-65Hc :	C-----	-----	-----	-----	-----	: 356
PtIP-65Hd :	C-----	-----	-----	-----	-----	: 358

Fig 18

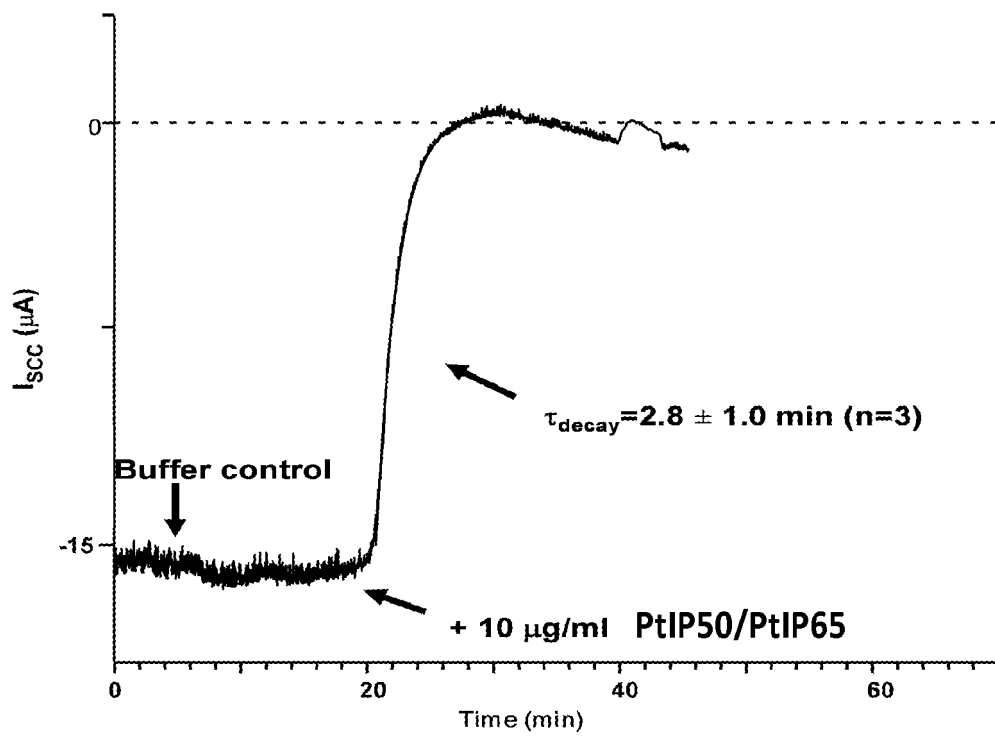


Fig 19

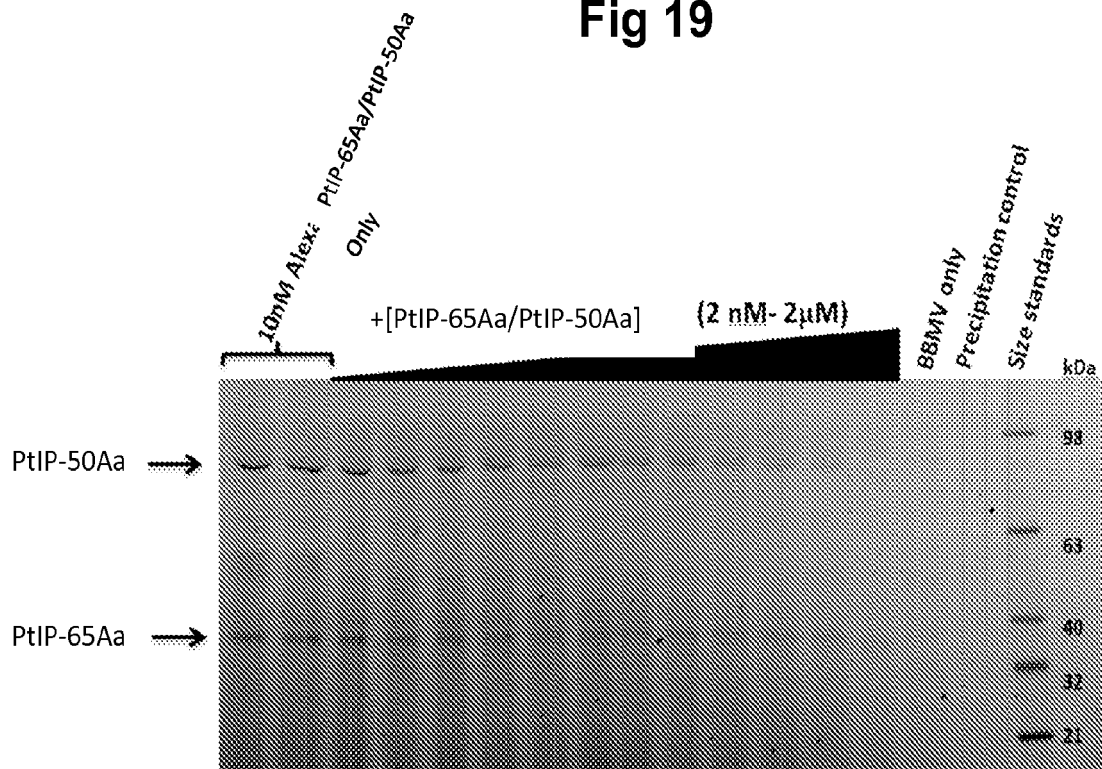


Fig 20a

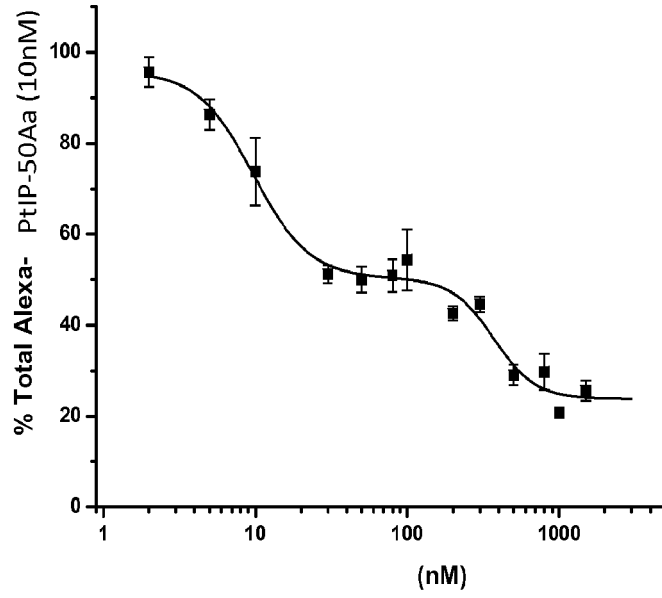
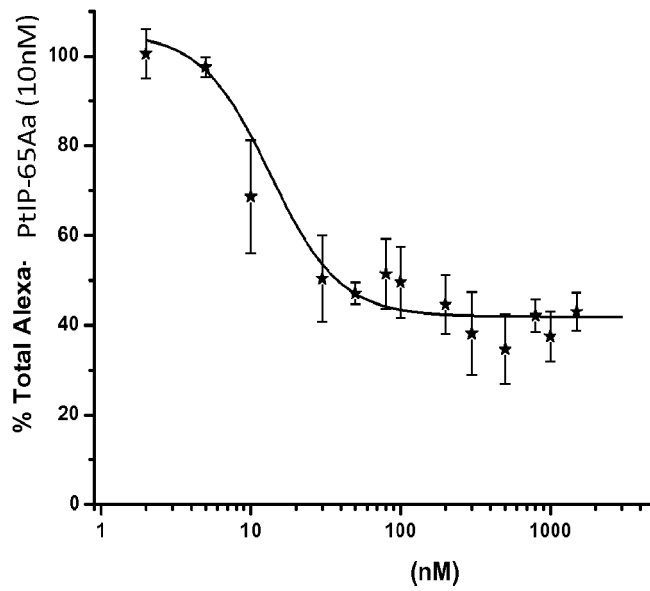


Fig 20b



REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO 2013098858 A2 [0006]
- US 6048838 A [0026]
- US 6379946 B [0026]
- US 792861 [0026]
- US 800233 [0026]
- US 839702 [0026]
- US 5880275 A [0027]
- US 7858849 B [0027]
- US 8304604 B [0027]
- US 8304605 B [0027]
- US 8476226 B [0027]
- US 525318 [0027]
- US 6033874 A [0027]
- US 5188960 A [0027]
- US 6218188 B [0027]
- US 7070982 B [0027]
- US 6962705 B [0027]
- US 6713063 B [0027]
- US 7064249 B [0027]
- US 20100017914 [0027]
- US 7329736 B [0027]
- US 7449552 B [0027]
- US 7803943 B [0027]
- US 7476781 B [0027]
- US 7105332 B [0027]
- US 7378499 B [0027]
- US 7462760 B [0027]
- US 6127180 A [0027]
- US 6624145 B [0027]
- US 6340593 B [0027]
- US 6248535 B [0027]
- US 6326351 B [0027]
- US 6399330 B [0027]
- US 6949626 B [0027]
- US 7385107 B [0027]
- US 7504229 B [0027]
- US 20060191034 A [0027]
- US 20120278954 A [0027]
- WO 2012139004 A [0027]
- US 6083499 A [0027]
- US 6548291 B [0027]
- US 20080295207 [0027]
- US 2006033867 W [0027]
- US 8236757 B [0027]
- US 7923602 B [0027]
- WO 2006083891 A [0027]
- WO 2005038032 A [0027]
- WO 2005021585 A [0027]
- US 20040250311 [0027]
- US 20040216186 [0027]
- US 20040210965 [0027]
- US 20040210964 A [0027]
- US 20040197917 [0027]
- US 20040197916 [0027]
- WO 2006119457 A [0027]
- WO 2004074462 A [0027]
- US 8084416 B [0027]
- US 20110023184 [0027]
- US 20110263488 [0027]
- US 20100197592 [0027]
- WO 2011103248 A [0027]
- WO 2011103247 A [0027]
- US 8334431 B [0027]
- US 20100298211 [0027]
- US 20090144852 [0027]
- US 8318900 B [0027]
- US 20100005543 [0027]
- US 8319019 B [0027]
- US 20110064710 [0027]
- US 20120317682 A [0027]
- US 20120311746 A [0027]
- US 20120311745 A [0027]
- US 20120317681 A [0027]
- US 20120331590 A [0027]
- US 20120331589 A [0027]
- US 20120324606 A [0027]
- US 20120324605 A [0027]
- US 20130167269 A [0027]
- US 20130167268 A [0027]
- US 20130116170 A [0027]
- US 7491869 B [0027]
- US 5877012 A [0027]
- US 6107279 A [0027]
- US 6137033 A [0027]
- US 7244820 B [0027]
- US 7615686 B [0027]
- US 8237020 B [0027]
- US 7491698 B [0027]
- US 8084418 B [0027]
- US 8334366 B [0027]
- US 5500365 A [0035] [0057]
- US 5689052 A [0035] [0057]
- US 6365377 B [0078]
- US 6531316 B [0078]
- US 5723323 PCT [0097]
- US 5763192 PCT [0097]
- US 5814476 PCT [0097]
- US 5817483 PCT [0097]

EP 3 102 684 B1

- US 5824514 PCT [0097]
- US 5976862 PCT [0097]
- US 5605793 PCT [0097]
- US 5811238 PCT [0097]
- US 5830721 PCT [0097]
- US 5834252 PCT [0097]
- US 5837458 PCT [0097]
- WO 199522625 PCT [0097]
- WO 199633207 PCT [0097]
- WO 199720078 PCT [0097]
- WO 199735966 PCT [0097]
- WO 199941402 PCT [0097]
- WO 199941383 PCT [0097]
- WO 199941369 PCT [0097]
- WO 199941368 PCT [0097]
- EP 752008 PCT [0097]
- EP 0932670 PCT [0097]
- WO 199923107 PCT [0097]
- WO 199921979 PCT [0097]
- WO 199831837 PCT [0097]
- WO 199827230 PCT [0097]
- WO 200000632 PCT [0097]
- WO 200009679 PCT [0097]
- WO 199842832 PCT [0097]
- WO 199929902 PCT [0097]
- WO 199841653 PCT [0097]
- WO 199841622 PCT [0097]
- WO 199842727 PCT [0097]
- WO 200018906 PCT [0097]
- WO 200004190 PCT [0097]
- WO 200042561 PCT [0097]
- WO 200042559 PCT [0097]
- WO 200042560 PCT [0097]
- WO 200123401 PCT [0097]
- US 0106775 W [0097]
- US 5576195 A [0175]
- US 5846818 A [0175]
- US 7193133 B [0175]
- US 20070277263 A [0178]
- US 4554101 A [0188] [0189]
- US 5605793 A [0191]
- US 5837458 A [0191]
- US 20090144863 [0200]
- US 7803992 B [0200]
- US 5380831 A [0203] [0210]
- US 5436391 A [0203]
- US 200909044298 [0208]
- US 20120304336 [0209]
- WO 199943838 A [0212] [0222]
- US 6072050 A [0212] [0222]
- US 5659026 A [0212]
- US 5608149 A [0212] [0222]
- US 5608144 A [0212] [0222]
- US 5604121 A [0212] [0222]
- US 5569597 A [0212] [0222]
- US 5466785 A [0212] [0222]
- US 5399680 A [0212] [0222]
- US 5268463 A [0212] [0222]
- US 5608142 A [0212] [0222]
- US 6177611 B [0212] [0222]
- US 5428148 A [0213]
- WO 199943819 A [0214]
- US 5750386 A [0215] [0219]
- US 5814618 A [0216]
- US 5789156 A [0216]
- US 5837876 A [0219]
- US 5633363 A [0219]
- US 5459252 A [0219]
- US 5401836 A [0219]
- US 5110732 A [0219]
- US 5023179 A [0219]
- US 20130117883 A [0219]
- US 6225529 B [0220]
- WO 200012733 A [0220]
- US 7294760 B [0220]
- US 7847153 B [0220]
- US 004357 [0224]
- US 10427692 B [0224]
- US 5563055 A [0228]
- US 5981840 A [0228] [0330]
- US 4945050 A [0228] [0331]
- US 5879918 A [0228]
- US 5886244 A [0228]
- US 5932782 A [0228]
- WO 0028058 A [0228]
- US 5240855 A [0228]
- US 5322783 A [0228]
- US 5324646 A [0228]
- US 5736369 A [0228]
- WO 199925821 A [0230] [0247]
- WO 199925854 A [0230] [0247]
- WO 199925840 A [0230] [0247]
- WO 199925855 A [0230] [0247]
- WO 199925853 A [0230] [0247]
- US 5889191 A [0235]
- US 5889190 A [0235]
- US 5866785 A [0235]
- US 5589367 A [0235]
- US 5316931 A [0235]
- US 5039523 A [0251]
- EP 0480762 A2 [0251]
- EP 0192319 A [0260]
- US 6468523 B [0265]
- US 5743477 A [0282]
- WO 199832326 A [0330]
- US 20030120054 A [0349]
- WO 2003018810 A [0349]

Non-patent literature cited in the description

- **A. R. SMITH et al.** *TAXON*, 2006, vol. 55, 705-731 [0023] [0039] [0060] [0147]
- **MONALYSIN.** *PLoS Pathogens*, 2011, vol. 7, 1-13 [0026]
- **PECHY-TARR.** *Environmental Microbiology*, 2008, vol. 10, 2368-2386 [0026]
- **LIU et al.** *J. Agric. Food Chem.*, 2010, vol. 58, 12343-12349 [0026]
- **ZHANG et al.** *Annals of Microbiology*, 2009, vol. 59, 45-50 [0026]
- **LI et al.** *Plant Cell Tiss. Organ Cult.*, 2007, vol. 89, 159-168 [0026]
- **HINCHLIFFE et al.** *The Open Toxicology Journal*, 2010, vol. 3, 101-118 [0026]
- **MORGAN et al.** *Applied and Envir. Micro.*, 2001, vol. 67, 2062-2069 [0026]
- **NAIMOV et al.** *Applied and Environmental Microbiology*, 2008, vol. 74, 7145-7151 [0027]
- **CRICKMORE et al.** *Bacillus thuringiensis toxin nomenclature*, 2011 [0027]
- **VAN FRANKENHUYZEN.** *J. Invert. Path.*, 2009, vol. 101, 1-16 [0027]
- **SANAHUJA.** *Plant Biotech Journal*. 2011, vol. 9, 283-300 [0027]
- **PURCELL et al.** *Biochem Biophys Res Commun*, 1993, vol. 15, 1406-1413 [0027]
- **SCHUETTPELZ E. ; PRYER K. M.** *TAXON*, 2007, vol. 56, 1037-1050 [0039] [0051] [0060] [0147]
- **N. WIKSTROM.** *American Fern Journal*, 2001, vol. 91, 150-156 [0051]
- **KARLIN ; ALTSCHUL.** *Proc. Natl. Acad. Sci. USA*, 1990, vol. 87, 2264 [0083]
- **KARLIN ; ALTSCHUL.** *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90, 5873-5877 [0083]
- **ALTSCHUL et al.** *J. Mol. Biol.*, 1990, vol. 215, 403 [0083]
- **ALTSCHUL et al.** *Nucleic Acids Res.*, 1997, vol. 25, 3389 [0083]
- **HIGGINS et al.** *Nucleic Acids Res.*, 1994, vol. 22, 4673-4680 [0084]
- **MYERS ; MILLER.** *CABIOS*, 1988, vol. 4, 11-17 [0084]
- **NEEDLEMAN ; WUNSCH.** *J. Mol. Biol.*, 1970, vol. 48 (3), 443-453 [0085]
- **SOONG et al.** *Nat Genet*, 2000, vol. 25 (4), 436-439 [0094]
- **STEMMER et al.** *Tumor Targeting*, 1999, vol. 4, 1-4 [0094]
- **NESS et al.** *Nat Biotechnol*, 1999, vol. 17, 893-896 [0094]
- **CHANG et al.** *Nat Biotechnol*, 1999, vol. 17, 793-797 [0094]
- **MINSHULL ; STEMMER.** *Curr Opin Chem Biol*, 1999, vol. 3, 284-290 [0094]
- **CHRISTIANS et al.** *Nat Biotechnol*, 1999, vol. 17, 259-264 [0094]
- **CRAMERI et al.** *Nature*, 1998, vol. 391, 288-291 [0094] [0191]
- **CRAMERI et al.** *Nat Biotechnol*, 1997, vol. 15, 436-438 [0094]
- **ZHANG et al.** *PNAS*, 1997, vol. 94, 4504-4509 [0094]
- **PATTEN et al.** *Curr Opin Biotechnol*, 1997, vol. 8, 724-733 [0094]
- **CRAMERI et al.** *Nat Med*, 1996, vol. 2, 100-103 [0094]
- **CRAMERI et al.** *Nat Biotechnol*, 1996, vol. 14, 315-319 [0094]
- **GATES et al.** *J Mol Biol*, 1996, vol. 255, 373-386 [0094]
- Sexual PCR and Assembly PCR. **STEMMER.** *The Encyclopedia of Molecular Biology*. VCH Publishers, 1996, 447-457 [0094]
- **CRAMERI ; STEMMER.** *BioTechniques*, 1995, vol. 18, 194-195 [0094]
- **STEMMER et al.** *Gene*, 1995, vol. 164, 49-53 [0094] [0175]
- **STEMMER.** *Science*, 1995, vol. 270, 1510 [0094]
- **STEMMER.** *Bio/Technology*, 1995, vol. 13, 549-553 [0094]
- **STEMMER.** *Nature*, 1994, vol. 370, 389-391 [0094] [0191]
- **STEMMER.** *PNAS*, 1994, vol. 91, 10747-10751 [0094]
- **LING et al.** *Anal Biochem*, 1997, vol. 254 (2), 157-178 [0095]
- **DALE et al.** *Methods Mol Biol*, 1996, vol. 57, 369-374 [0095]
- **SMITH.** *Ann Rev Genet*, 1985, vol. 19, 423-462 [0095]
- **BOTSTEIN ; SHORTLE.** *Science*, 1985, vol. 229, 1193-1201 [0095]
- **CARTER.** *Biochem J*, 1986, vol. 237, 1-7 [0095]
- The efficiency of oligonucleotide directed mutagenesis. **KUNKEL.** *Nucleic Acids & Molecular Biology*. Springer Verlag, 1987 [0095]
- **KUNKEL.** *PNAS*, 1985, vol. 82, 488-492 [0095]
- **KUNKEL et al.** *Methods Enzymol*, 1987, vol. 154, 367-382 [0095]
- **BASS et al.** *Science*, 1988, vol. 242, 240-245 [0095]
- **ZOLLER ; SMITH.** *Methods Enzymol*, 1983, vol. 100, 468-500 [0095]
- **ZOLLER ; SMITH.** *Methods Enzymol*, 1987, vol. 154, 329-350 [0095]
- **ZOLLER ; SMITH.** *Nucleic Acids Res*, 1982, vol. 10, 6487-6500 [0095]
- **TAYLOR et al.** *Nucl Acids Res*, 1985, vol. 13, 8749-8764 [0095]
- **TAYLOR et al.** *Nucl Acids Res*, 1985, vol. 13, 8765-8787 [0095]
- **NAKAMAYE ; ECKSTEIN.** *Nucl Acids Res*, 1986, vol. 14, 9679-9698 [0095]

- **SAYERS et al.** *Nucl Acids Res*, 1988, vol. 16, 791-802 [0095]
- **SAYERS et al.** *Nucl Acids Res*, 1988, vol. 16, 803-814 [0095]
- **KRAMER et al.** *Nucl Acids Res*, 1984, vol. 12, 9441-9456 [0095]
- **KRAMER ; FRITZ.** *Methods Enzymol*, 1987, vol. 154, 350-367 [0095]
- **KRAMER et al.** *Nucl Acids Res*, 1988, vol. 16, 7207 [0095]
- **FRITZ et al.** *Nucl Acids Res*, 1988, vol. 16, 6987-6999 [0095]
- **KRAMER et al.** *Cell*, 1984, vol. 38, 879-887 [0096]
- **CARTER et al.** *Nucl Acids Res*, 1985, vol. 13, 4431-4443 [0096]
- **CARTER.** *Methods in Enzymol*, 1987, vol. 154, 382-403 [0096]
- **EGHTEDARZADEH ; HENIKOFF.** *Nucl Acids Res*, 1986, vol. 14, 5115 [0096]
- **WELLS et al.** *Phil Trans R Soc Lond A*, 1986, vol. 317, 415-423 [0096]
- **NAMBIAR et al.** *Science*, 1984, vol. 223, 1299-1301 [0096]
- **SAKAMAR ; KHORANA.** *Nucl Acids Res*, 1988, vol. 14, 6361-6372 [0096]
- **WELLS et al.** *Gene*, vol. 34, 315-323 [0096]
- **GRUNDSTROM et al.** *Nucl Acids Res*, 1985, vol. 13, 3305-3316 [0096]
- **MANDECKI.** *PNAS*, 1986, vol. 83, 7177-7181 [0096]
- **ARNOLD.** *Curr Opin Biotech*, 1993, vol. 4, 450-455 [0096]
- *Methods Enzymol*, vol. 154 [0096]
- **SAMBROOK et al.** *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, 1989 [0099] [0103] [0106]
- *PCR Protocols: A Guide to Methods and Applications*. Academic Press, 1990 [0099]
- *PCR Strategies*. Academic Press, 1995 [0099]
- *PCR Methods Manual*. Academic Press, 1999 [0099]
- **SCOTT PATTERSON.** *Current Protocol in Molecular Biology*. John Wiley & Son Inc, 1998, vol. 10.22, 1-24 [0101]
- **MEINKOTH ; WAHL.** *Anal. Biochem.*, 1984, vol. 138, 267-284 [0106]
- **TIJSSEN.** *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes*. Elsevier, 1993 [0106]
- *Current Protocols in Molecular Biology*. Greene Publishing, 1995 [0106]
- **LARKIN M.A et al.** *Bioinformatics*, 2007, vol. 23 (21), 2947-2948 [0142]
- **BAILEY T.L. ; ELKAN C.** *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*. AAAI Press, 1994, 28-36 [0143]
- **JONES DT.** *J. Mol. Biol.*, 1999, vol. 292, 195-202 [0144]
- **FUCHS, R.L. ; J.D. ASTWOOD.** *Food Technology*, 1996, vol. 50, 83-88 [0146] [0166]
- **ASTWOOD, J.D. et al.** *Nature Biotechnology*, 1996, vol. 14, 1269-1273 [0146] [0166]
- **FU TJ et al.** *J. Agric Food Chem.*, 2002, vol. 50, 7154-7160 [0146] [0166]
- **COMAI et al.** *J. Biol. Chem.*, 1988, vol. 263 (29), 15104-9 [0175]
- **DAYHOFF et al.** *Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found. 1978* [0185]
- **KYTE ; DOOLITTLE.** *J Mol Biol.*, 1982, vol. 157 (1), 105-32 [0186]
- **STEMMER.** *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, 10747-10751 [0191]
- **CRAMERI et al.** *Nature Biotech.*, 1997, vol. 15, 436-438 [0191]
- **MOORE et al.** *J. Mol. Biol.*, 1997, vol. 272, 336-347 [0191]
- **ZHANG et al.** *Proc. Natl. Acad. Sci. USA*, 1997, vol. 94, 4504-4509 [0191]
- **NAIMOV et al.** *Appl. Environ. Microbiol.*, 2001, vol. 67, 5328-5330 [0192]
- **DE MAAGD et al.** *Appl. Environ. Microbiol.*, 1996, vol. 62, 1537-1543 [0192]
- **GE et al.** *J. Biol. Chem.*, 1991, vol. 266, 17954-17958 [0192]
- **SCHNEPF et al.** *J. Biol. Chem.*, 1990, vol. 265, 20923-20930 [0192]
- **RANG et al.** *Appl. Environ. Microbiol.*, vol. 65, 2918-2925 [0192]
- **GALLIE et al.** *Molecular Biology of RNA*. Liss, 1989, 237-256 [0200] [0207]
- **GALLIE et al.** *Gene*, 1987, vol. 60, 217-25 [0200]
- **BENFEY et al.** *EMBO J.*, 1990, vol. 9, 1685-96 [0200]
- **GUERINEAU et al.** *Mol. Gen. Genet.*, 1991, vol. 262, 141-144 [0202]
- **PROUDFOOT.** *Cell*, 1991, vol. 64, 671-674 [0202]
- **SANFACON et al.** *Genes Dev.*, 1991, vol. 5, 141-149 [0202]
- **MOGEN et al.** *Plant Cell*, 1990, vol. 2, 1261-1272 [0202]
- **MUNROE et al.** *Gene*, 1990, vol. 91, 151-158 [0202]
- **BALLAS et al.** *Nucleic Acids Res.*, 1989, vol. 17, 7891-7903 [0202]
- **JOSHI et al.** *Nucleic Acid Res.*, 1987, vol. 15, 9627-9639 [0202]
- **CAMPBELL ; GOWRI.** *Plant Physiol.*, 1990, vol. 92, 1-11 [0203]
- **MURRAY et al.** *Nucleic Acids Res.*, 1989, vol. 17, 477-498 [0203]
- **LIU H et al.** *Mol Bio Rep*, 2010, vol. 37, 677-684 [0203]
- **ELROY-STEIN et al.** *Proc. Natl. Acad. Sci. USA*, 1989, vol. 86, 6126-6130 [0207]
- **GALLIE et al.** *Gene*, 1995, vol. 165 (2), 233-238 [0207]
- **MACEJAK et al.** *Nature*, 1991, vol. 353, 90-94 [0207]
- **JOBLING et al.** *Nature*, 1987, vol. 325, 622-625 [0207]

- LOMMEL et al. *Virology*, 1991, vol. 81, 382-385 [0207]
- DELLA-CIOPPA et al. *Plant Physiol.*, 1987, vol. 84, 965-968 [0207]
- CHANG. *Methods Enzymol.*, 1987, vol. 153, 507-516 [0208]
- KIESELBACH et al. *FEBS LETT*, 2000, vol. 480, 271-276 [0208]
- PELTIER et al. *Plant Cell*, 2000, vol. 12, 319-341 [0208]
- BRICKER et al. *Biochim. Biophys Acta*, 2001, vol. 1503, 350-356 [0208]
- KIESELBACH et al. *Photosynthesis Research*, 2003, vol. 78, 249-264 [0208]
- GOFF et al. *Science*, 2002, vol. 296, 92-100 [0208]
- ODELL et al. *Nature*, 1985, vol. 313, 810-812 [0212]
- MCELROY et al. *Plant Cell*, 1990, vol. 2, 163-171 [0212]
- CHRISTENSEN et al. *Plant Mol. Biol.*, 1989, vol. 12, 619-632 [0212]
- CHRISTENSEN et al. *Plant Mol. Biol.*, 1992, vol. 18, 675-689 [0212]
- LAST et al. *Theor. Appl. Genet.*, 1991, vol. 81, 581-588 [0212]
- VELTEN et al. *EMBO J.*, 1984, vol. 3, 2723-2730 [0212]
- RYAN. *Ann. Rev. Phytopath.*, 1990, vol. 28, 425-449 [0213]
- DUAN et al. *Nature Biotechnology*, 1996, vol. 14, 494-498 [0213]
- STANFORD et al. *Mol. Gen. Genet.*, 1989, vol. 215, 200-208 [0213]
- MCGURL et al. *Science*, 1992, vol. 225, 1570-1573 [0213]
- ROHMEIER et al. *Plant Mol. Biol.*, 1993, vol. 22, 783-792 [0213]
- ECKELKAMP et al. *FEBS Letters*, 1993, vol. 323, 73-76 [0213]
- CORDEROK et al. *Plant J.*, 1994, vol. 6 (2), 141-150 [0213]
- REDOLFI et al. *Neth. J. Plant Pathol.*, 1983, vol. 89, 245-254 [0214]
- UKNES et al. *Plant Cell*, 1992, vol. 4, 645-656 [0214]
- VAN LOON. *Plant Mol. Virol.*, 1985, vol. 4, 111-116 [0214]
- MARINEAU et al. *Plant Mol. Biol.*, 1987, vol. 9, 335-342 [0215]
- MATTON et al. *Molecular Plant-Microbe Interactions*, 1989, vol. 2, 325-331 [0215]
- SOMSISCH et al. *Proc. Natl. Acad. Sci. USA*, 1986, vol. 83, 2427-2430 [0215]
- SOMSISCH et al. *Mol. Gen. Genet.*, 1988, vol. 2, 93-98 [0215]
- YANG. *Proc. Natl. Acad. Sci. USA*, 1996, vol. 93, 14972-14977 [0215]
- CHEN et al. *Plant J.*, 1996, vol. 10, 955-966 [0215]
- ZHANG et al. *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, 2507-2511 [0215]
- WARNER et al. *Plant J.*, 1993, vol. 3, 191-201 [0215]
- SIEBERTZ et al. *Plant Cell*, 1989, vol. 1, 961-968 [0215]
- CORDERO et al. *Physiol. Mol. Plant Pathol.*, 1992, vol. 41, 189-200 [0215]
- SCHENA et al. *Proc. Natl. Acad. Sci. USA*, 1991, vol. 88, 10421-10425 [0216]
- MCNELLIS et al. *Plant J.*, 1998, vol. 14 (2), 247-257 [0216]
- GATZ et al. *Mol. Gen. Genet.*, 1991, vol. 227, 229-237 [0216]
- YAMAMOTO et al. *Plant J.*, 1997, vol. 12 (2), 255-265 [0217] [0218]
- KAWAMATA et al. *Plant Cell Physiol.*, 1997, vol. 38 (7), 792-803 [0217]
- HANSEN et al. *Mol. Gen. Genet.*, 1997, vol. 254 (3), 337-343 [0217]
- RUSSELL et al. *Transgenic Res.*, 1997, vol. 6 (2), 157-168 [0217]
- RINEHART et al. *Plant Physiol.*, 1996, vol. 112 (3), 1331-1341 [0217]
- VAN CAMP et al. *Plant Physiol.*, 1996, vol. 112 (2), 525-535 [0217]
- CANEVASCINI et al. *Plant Physiol.*, 1996, vol. 112 (2), 513-524 [0217]
- YAMAMOTO et al. *Plant Cell Physiol.*, 1994, vol. 35 (5), 773-778 [0217] [0218]
- LAM. *Results Probl. Cell Differ.*, 1994, vol. 20, 181-196 [0217]
- OROZCO et al. *Plant Mol Biol.*, 1993, vol. 23 (6), 1129-1138 [0217]
- MATSUOKA et al. *Proc Natl. Acad. Sci. USA*, 1993, vol. 90 (20), 9586-9590 [0217]
- GUEVARA-GARCIA et al. *Plant J.*, 1993, vol. 4 (3), 495-505 [0217]
- KWON et al. *Plant Physiol.*, 1994, vol. 105, 357-67 [0218]
- GOTOR et al. *Plant J.*, 1993, vol. 3, 509-18 [0218]
- OROZCO et al. *Plant Mol. Biol.*, 1993, vol. 23 (6), 1129-1138 [0218]
- MATSUOKA et al. *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90 (20), 9586-9590 [0218]
- HIRE et al. *Plant Mol. Biol.*, 1992, vol. 20 (2), 207-218 [0219]
- KELLER ; BAUMGARTNER. *Plant Cell*, 1991, vol. 3 (10), 1051-1061 [0219]
- SANGER et al. *Plant Mol. Biol.*, 1990, vol. 14 (3), 433-443 [0219]
- MIAO et al. *Plant Cell*, 1991, vol. 3 (1), 11-22 [0219]
- BOGUSZ et al. *Plant Cell*, 1990, vol. 2 (7), 633-641 [0219]
- PLANT. *Science*, vol. 79 (1), 69-76 [0219]
- *EMBO J.*, vol. 8 (2), 343-350 [0219]
- KUSTER et al. *Plant Mol. Biol.*, 1995, vol. 29 (4), 759-772 [0219]
- CAPANA et al. *Plant Mol. Biol.*, 1994, vol. 25 (4), 681-691 [0219]

- THOMPSON et al. *BioEssays*, 1989, vol. 10, 108 [0220]
- JOFUKU ; GOLDBERG. *Plant Cell*, 1989, vol. 1, 1079-1093 [0220]
- HERRERA ESTRELLA et al. *EMBO J.*, 1983, vol. 2, 987-992 [0224]
- HERRERA ESTRELLA et al. *Nature*, 1983, vol. 303, 209-213 [0224]
- MEIJER et al. *Plant Mol. Biol.*, 1991, vol. 16, 807-820 [0224]
- JONES et al. *Mol. Gen. Genet.*, 1987, vol. 210, 86-91 [0224]
- BRETAGNE-SAGNARD et al. *Transgenic Res.*, 1996, vol. 5, 131-137 [0224]
- HILLE et al. *Plant Mol. Biol.*, 1990, vol. 7, 171-176 [0224]
- GUERINEAU et al. *Plant Mol. Biol.*, 1990, vol. 15, 127-136 [0224]
- STALKER et al. *Science*, 1988, vol. 242, 419-423 [0224]
- SHAW et al. *Science*, 1986, vol. 233, 478-481 [0224]
- DEBLOCK et al. *EMBO J.*, 1987, vol. 6, 2513-2518 [0224]
- YARRANTON. *Curr. Opin. Biotech.*, 1992, vol. 3, 506-511 [0224]
- CHRISTOPHERSON et al. *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, 6314-6318 [0224]
- YAO et al. *Cell*, 1992, vol. 71, 63-72 [0224]
- REZNIKOFF. *Mol. Microbiol.*, 1992, vol. 6, 2419-2422 [0224]
- BARKLEY et al. *The Operon*, 1980, 177-220 [0224]
- HU et al. *Cell*, 1987, vol. 48, 555-566 [0224]
- BROWN et al. *Cell*, 1987, vol. 49, 603-612 [0224]
- FIGGE et al. *Cell*, 1988, vol. 52, 713-722 [0224]
- DEUSCHLE et al. *Proc. Natl. Acad. Sci. USA*, 1989, vol. 86, 5400-5404 [0224]
- FUERST et al. *Proc. Natl. Acad. Sci. USA*, 1989, vol. 86, 2549-2553 [0224]
- DEUSCHLE et al. *Science*, 1990, vol. 248, 480-483 [0224]
- GOSSEN. *Ph.D. Thesis, University of Heidelberg*, 1993 [0224]
- REINES et al. *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90, 1917-1921 [0224]
- LABOW et al. *Mol. Cell. Biol.*, 1990, vol. 10, 3343-3356 [0224]
- ZAMBRETTI et al. *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, 3952-3956 [0224]
- BAIM et al. *Proc. Natl. Acad. Sci. USA*, 1991, vol. 88, 5072-5076 [0224]
- WYBORSKI et al. *Nucleic Acids Res.*, 1991, vol. 19, 4647-4653 [0224]
- HILLENAND-WISSMAN. *Topics Mol. Struc. Biol.*, 1989, vol. 10, 143-162 [0224]
- DEGENKOLB et al. *Antimicrob. Agents Chemother.*, 1991, vol. 35, 1591-1595 [0224]
- KLEINSCHNIDT et al. *Biochemistry*, 1988, vol. 27, 1094-1104 [0224]
- BONIN. *Ph.D. Thesis, University of Heidelberg*, 1993 [0224]
- GOSSEN et al. *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, 5547-5551 [0224]
- OLIVA et al. *Antimicrob. Agents Chemother.*, 1992, vol. 36, 913-919 [0224]
- HLAVKA et al. *Handbook of Experimental Pharmacology*. Springer-Verlag, 1985, vol. 78 [0224]
- GILL et al. *Nature*, 1988, vol. 334, 721-724 [0224]
- CROSSWAY et al. *Biotechniques*, 1986, vol. 4, 320-334 [0228]
- RIGGS et al. *Proc. Natl. Acad. Sci. USA*, 1986, vol. 83, 5602-5606 [0228]
- PASZKOWSKI et al. *EMBO J.*, 1984, vol. 3, 2717-2722 [0228]
- TOMES et al. *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*. Springer-Verlag, 1995 [0228]
- MCCABE et al. *Biotechnology*, 1988, vol. 6, 923-926 [0228]
- TU et al. *Plant Molecular Biology*, 1998, vol. 37, 829-838 [0228]
- CHONG et al. *Transgenic Research*, 2000, vol. 9, 71-78 [0228]
- WEISSINGER et al. *Ann. Rev. Genet.*, 1988, vol. 22, 421-477 [0228]
- SANFORD et al. *Particulate Science and Technology*, 1987, vol. 5, 27-37 [0228]
- CHRISTOU et al. *Plant Physiol.*, 1988, vol. 87, 671-674 [0228]
- MCCABE et al. *Bio/Technology*, 1988, vol. 6, 923-926 [0228]
- FINER ; MCMULLEN. *In Vitro Cell Dev. Biol.*, 1991, vol. 27P, 175-182 [0228]
- SINGH et al. *Theor. Appl. Genet.*, 1998, vol. 96, 319-324 [0228]
- DATTA et al. *Biotechnology*, 1990, vol. 8, 736-740 [0228]
- KLEIN et al. *Proc. Natl. Acad. Sci. USA*, 1988, vol. 85, 4305-4309 [0228]
- KLEIN et al. *Biotechnology*, 1988, vol. 6, 559-563 [0228]
- KLEIN et al. *Plant Physiol.*, 1988, vol. 91, 440-444 [0228]
- FROMM et al. *Biotechnology*, 1990, vol. 8, 833-839 [0228]
- HOOYKAAS-VAN SLOGTEREN et al. *Nature*, 1984, vol. 311, 763-764 [0228]
- BYTEBIER et al. *Proc. Natl. Acad. Sci. USA*, 1987, vol. 84, 5345-5349 [0228]
- DE WET et al. *The Experimental Manipulation of Ovule Tissues*. Longman, 1985, 197-209 [0228]
- KAEPLER et al. *Plant Cell Reports*, 1990, vol. 9, 415-418 [0228]
- KAEPLER et al. *Theor. Appl. Genet.*, 1992, vol. 84, 560-566 [0228]
- D'HALLUIN et al. *Plant Cell*, 1992, vol. 4, 1495-1505 [0228]

- **LI et al.** *Plant Cell Reports*, 1993, vol. 12, 250-255 [0228]
- **CHRISTOU ; FORD.** *Annals of Botany*, 1995, vol. 75, 407-413 [0228]
- **OSJODA et al.** *Nature Biotechnology*, 1996, vol. 14, 745-750 [0228]
- **CROSSWAY et al.** *Mol Gen. Genet.*, 1986, vol. 202, 179-185 [0229]
- **NOMURA et al.** *Plant Sci.*, 1986, vol. 44, 53-58 [0229]
- **HEPLER et al.** *Proc. Natl. Acad. Sci.*, 1994, vol. 91, 2176-2180 [0229]
- **HUSH et al.** *The Journal of Cell Science*, 1994, vol. 107, 775-784 [0229]
- **HELLENS ; MULLINEAUX.** *Trends in Plant Science*, 2000, vol. 5, 446-451 [0231]
- **HIEI et al.** *The Plant Journal*, 1994, vol. 6, 271-282 [0233]
- **ISHIDA et al.** *Nature Biotechnology*, 1996, vol. 14, 745-750 [0233]
- **AYRES ; PARK.** *Critical Reviews in Plant Science*, 1994, vol. 13, 219-239 [0233]
- **BOMMINENI ; JAUHAR.** *Maydica*, 1997, vol. 42, 107-120 [0233]
- **MCCORMICK et al.** *Plant Cell Reports*, 1986, vol. 5, 81-84 [0234]
- **SVAB et al.** *Proc. Natl. Acad. Sci. USA*, 1990, vol. 87, 8526-8530 [0236]
- **SVAB ; MALIGA.** *Proc. Natl. Acad. Sci. USA*, 1993, vol. 90, 913-917 [0236]
- **SVAB ; MALIGA.** *EMBO J.*, 1993, vol. 12, 601-606 [0236]
- **MCBRIDE et al.** *Proc. Natl. Acad. Sci. USA*, 1994, vol. 91, 7301-7305 [0236]
- **SAMBROOK ; RUSSELL.** *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, 2001 [0243]
- **HANDELSMAN et al.** *Appl. Environ. Microbiol.*, 1991, vol. 56, 713-718 [0255]
- **LERECIUS et al.** *FEMS Microbiol. Letts.*, 1989, vol. 60, 211-218 [0256]
- **GHRAYEB et al.** *EMBO J*, 1984, vol. 3, 2437-2442 [0258]
- **DUFFAUD et al.** *Meth. Enzymol.*, 1987, vol. 153, 492 [0258]
- **GAERTNER et al.** *Advanced Engineered Pesticides.* 1993 [0259]
- **CZAPLA ; LANG.** *J. Econ. Entomol.*, 1990, vol. 83, 2480-2485 [0282]
- **ANDREWS et al.** *Biochem. J.*, 1988, vol. 252, 199-206 [0282]
- **MARRONE et al.** *J. of Economic Entomology*, 1985, vol. 78, 290-293 [0282]
- *The Pesticide Manual: A World Compendium.* the British Crop Production Council [0284]
- *Phil. Trans. R. Soc. Lond. B. The Royal Society*, 1998, vol. 353, 1777-1786 [0295]
- **ALTSCHUL et al.** *J. Mol. Biol.*, 1993, vol. 215, 403-410 [0316] [0322]
- **DAV.** *Plant Mol. Biol.*, 1999, vol. 40, 771-782 [0324]
- **NORRIS SR et al.** *Plant Mol Biol.*, 1993, vol. 21 (5), 895-906 [0324]
- **KAPILA.** *Plant Science*, 1997, vol. 122, 101-108 [0324]
- **PATTERSON.** *Current Protocol in Molecular Biology.* John Wiley & Son Inc, 1998, vol. 10, 1-24 [0324]
- **KLEIN et al.** *Nature*, 1987, vol. 327, 70-73 [0331]
- **MURASHIGE ; SKOOG.** *Physiol. Plant.*, 1962, vol. 15, 473 [0351]