

IMMUNOGENIC PROTEINS FROM THE DOMESTIC MITE, *Blomia Tropicalis*

FIELD OF THE INVENTION

5 The present invention relates generally to novel protein molecules and to derivatives, homologs, analogues, chemical equivalents and mimetics thereof capable of inducing, upregulating or otherwise facilitating the induction of an immune response to a mite and, more particularly, a mite from the family *Glycyphagidae*. These mites include dust mites and storage mites. The present invention also contemplates genetic sequences encoding
10 said protein molecules and derivatives, homologs, analogues, chemical equivalents and mimetics thereof. The present invention further provides genetic vaccines and other compositions comprising nucleic acid molecules. The molecules of the present invention are useful, *inter alia*, in a range of therapeutic, prophylactic and diagnostic applications.

15 BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

20 The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia or any other country.

House dust or storage mite is one of the most important sources of indoor allergens and
25 their role in triggering allergic diseases has been established (1,2). Besides two predominant mite species, *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df), *Blomia tropicalis* (Bt) is equally important in sensitizing allergic patients in tropical and subtropical areas of the world. Although allergens of *B. tropicalis* are considered as a major component of dust in Singapore (3), there is no significant
30 differences in sensitization pattern of mite sensitized population here (4). However, the importance of including *B. tropicalis* allergens in the diagnostic and therapeutic reagents is

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readily shown by a cross-reactivity study which showed there is very limited cross-reactivity between these two family of mites (5). This pyroglyphid mite species has been well documented to induce allergic asthma and allergic rhinitis (6-10). Epidemiological studies clearly indicated that Dp and Bt mites from the Pyroglyphidae and Glycyphagidae respectively, are the most common and important clinical mite species in the tropical and sub-tropical regions of the world (11,12,13). Thus, there are allergens which remain unknown and have to be characterized in *B. tropicalis*.

Studies on the allergenicity of *B. tropicalis* revealed the presence of more than 20 allergens. Crossed radioimmuno-electrophoresis (CRIE) studies identified 21 different species-specific IgE binding-antigens in *B. tropicalis* (14) while immunoblotting techniques were able to reveal the presence of 25 allergens with molecular weights ranging from 11 to 85 kD (9). Despite these initial findings, however, only three published Bt molecules purported to be allergens had been previously identified and characterized. Blo t 5, the first Bt allergen to be cloned with 40% amino acid homology to Der p 5, contains 432 bp open reading frame (15). To date, Blo t 5 is the only major allergen isolated from *B. tropicalis* with 70% IgE reactivity to mite allergic sera (16,17). Two other Bt allergens, Bt 6 (now identified as Bt13) and Bt 11a (now identified as Bt12), exhibited human IgE reactivity of 11% (18) and 50% (19), respectively. Bt 13 is composed of 934 bp with 390 bp open reading frame which codes for a 130 amino acid allergen with a molecular weight of 14.8 kD (18). On the other hand, Bt 12 is composed of 582 bp with 432 bp reading frame coding for a 20-residue putative signal peptide and a 124-residue mature protein with an estimated molecular weight of 14.206 kD (19). Thus far, the reported Bt allergens are low molecular weight proteins. Recent studies in other species of mites demonstrated the presence of high molecular weight allergens with significant IgE-binding properties from mite sensitive patients (20). Df 11 isolated from *Dermatophagoides farinae* is a 98 kD paramyosin allergen with a human IgE reactivity of 82% (21) while Mag 3 (M-177) isolated from *Dermatophagoides pteronyssinus* is a 177 kD allergen with a human IgE reactivity of 70% (22).

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To date, crude mite extract has been the sole diagnostic and therapeutic reagent for mite allergies. Due to poor extraction methodology and the incidence of protein degradation, it has not been possible to determine a complete profile of the immunogenic regions and/or specific epitopes of the dust or storage mite. This has resulted in poor diagnosis and immunotherapy of conditions such as mite induced allergies. Accordingly, there is a need to identify immunogenic regions and/or epitopes of the dust or storage mite in order to facilitate better diagnosis and immunotherapy of conditions related to the dust or storage mite, such as allergies.

- 10 In work leading up to the present invention, the inventors have studied the domestic mite (*B. tropicalis*) and have identified and sequenced novel immunogenic protein molecules. The identification of these molecules facilitates, *inter alia*, the development of more efficacious clinical reagents for the treatment and diagnosis of conditions involving the induction of an immune response to the mite, such as allergies. The allergens identified in accordance with the present invention are referred to herein as "Bt" allergens. Specific Bt allergens are "Bt11", "Bt10", "Bt5" and "BtA2". Insofar as "Bt5" is part of the invention, it is a polymorphic variant of the Bt5, as published by Arruda *et al.* (7), and consisting of the amino acid sequence and corresponding nucleotide sequence set forth in SEQ ID NO:36.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the
5 inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1,
10 <400>2, etc. A sequence listing is provided after the claims.

One aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from *B. tropicalis* or a derivative, homologue or
15 mimetic of said protein allergen.

Another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding protein allergen from a mite or a derivative, homologue or mimetic of
20 said protein allergen wherein said protein allergen is selected from Bt11, Bt10 and BtA2, each as herein defined, or polymorphic variants thereof and a polymorphic variant of Bt5 as herein defined.

A further aspect of the present invention provides an isolated nucleic acid molecule or
25 derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is Bt11 having an amino acid sequence as set forth in any one of SEQ ID NO:2 to SEQ ID NO:14 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to an amino acid
30 sequence set forth in in SEQ ID NO:2 to SEQ ID NO:14.

Still another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is Bt11 wherein said nucleotide
5 sequence is as substantially set forth in SEQ ID NO:1 or SEQ ID NO: 2 or SEQ ID NO:13 or a derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a complementary form thereof under low stringency conditions.

10 Yet another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is Bt10 having an amino acid sequence as substantially set forth in SEQ ID NO:29 or SEQ ID NO: 31 or a derivative, homologue
15 or mimetic thereof having at least about 45% similarity to amino acid sequences set forth in SEQ ID NO:29 or SEQ ID NO:31.

Still another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a
20 sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is Bt10 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:28 or SEQ ID NO: 30 or a nucleotide sequence having at least 45% similarity to SEQ ID NO:28 or SEQ ID NO:30 or a nucleotide sequence capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or a
25 complementary form thereof under low stringency conditions.

Even still another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative,
30 homologue or mimetic of said protein allergen, wherein said protein allergen is a polymorphic variant of Bt5 having an amino acid sequence as set forth in any one of SEQ

ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in any one of SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71.

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Even yet another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is a polymorphic variant of Bt5 wherein
10 said nucleotide sequence is substantially as set forth in any one of SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a derivative, homologue or mimetic thereof or a nucleotide sequence having at least about 45% similarity to SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a nucleotide sequence capable of hybridizing to any one of SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID
15 NO:67 or a complementary form thereof under low stringency conditions.

Another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of
20 said protein allergen, wherein said protein allergen is BtA2 having an amino acid sequence as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to an amino acid sequence set forth in SEQ ID NO:75.

25 A further aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is BtA2 wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:74 or a derivative, homologue or
30 mimetic thereof or a nucleotide sequence having at least about 45% similarity to SEQ ID NO:74 or a nucleotide sequence capable of hybridizing to SEQ ID NO:74.

Yet another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is selected from Bt11, Bt10 or BtA2, each as herein defined, or polymorphic variants thereof and a polymorphic variant of Bt5 as herein defined.

Still another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is Bt11, said amino acid sequence substantially as set forth in SEQ ID NO:3 to SEQ ID NO:12 or SEQ ID NO:14 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:3 to SEQ ID NO:12 or SEQ ID NO:14.

Even still another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is Bt11, said amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a complementary form thereof under low stringency conditions.

Even still another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is Bt10, said amino acid sequence substantially as set forth in SEQ ID NO:28 or SEQ ID NO:31 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:28 or SEQ ID NO:31.

Even yet another another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is Bt10, said amino acid sequence encoded by the nucleotide

sequene set forth in SEQ ID NO:28 or SEQ ID NO:30 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or a complementary form thereof under low stringency conditions.

5 Another another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is a polymorphic variant of Bt5, said amino acid sequence substantially as set forth in SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an
10 amino acid sequence set forth in SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71.

A further aspect another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen
15 wherein said protein allergen is a polymorphic variant of Bt5, said amino acid sequence encoded by the nucleotide sequene set forth in SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a complementary form thereof under low stringency conditions.

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Yet another another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is BtA2, said amino acid sequence substantially as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or having at least about
25 45% similarity to an amino acid sequence set forth in SEQ ID NO:75.

Still another another aspect of the present invention contemplates an isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is BtA2, said amino acid sequence encoded by the nucleotide
30 sequene set forth in SEQ ID NO:74 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:74 or a complementary form

thereof under low stringency conditions.

Table 1 is a list of single and three letter abbreviations used throughout the specification.

5 **Table 2** is a summary of amino acid and nucleotide sequence identifiers.

Table 3 summarizes the characteristics of the polymorphic residues found in Bt5 genes.

Table 4 is a summary of the results of human IgE plaque immunoassay (Example 13).

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Table 5 is a summary of the results of dot blot immunoassay (Example 13).

TABLE 1
Single and three letter amino acid abbreviations

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	The	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

TABLE 2

SEQUENCE IDENTIFIER	DESCRIPTION
SEQ ID NO:1	Nucleotide sequence of clone encoding Bt11
SEQ ID NO:2	Nucleotide and corresponding amino acid sequences of clone encoding Bt11
SEQ ID NO:3 to SEQ ID NO:12	Amino acid sequences encoded by SEQ ID NO:2
SEQ ID NO:13	Nucleotide sequence and corresponding amino acid sequence of <i>Bt11</i> gene
SEQ ID NO:14	Amino acid sequence of Bt11
SEQ ID NO:15	Amino acid sequence of <i>Dermatophagoides farinae</i> paramyosin
SEQ ID NO:16	Amino acid sequence of <i>Drosophila melanogaster</i> paramyosin
SEQ ID NO:17	Amino acid sequence of <i>Onchocerca volvulus</i> paramyosin
SEQ ID NO:18	Amino acid sequence of <i>Caenorhabditis elegans</i> paramyosin
SEQ ID NO:19	T3 primer
SEQ ID NO:20	T7 primer
SEQ ID NO:21	Gene-specific primer 1 (GSP1)
SEQ ID NO:22	Gene-specific primer 2 (GSP2)
SEQ ID NO:23	Nested universal primer (NUP)
SEQ ID NO:24	T7 primer
SEQ ID NO:25	Bt11 CS2f primer
SEQ ID NO:26	Bt11 R2 primer
SEQ ID NO:27	Bt11 polymorphic variant
SEQ ID NO:28	Nucleotide sequence of clone encoding Bt10
SEQ ID NO:29	Amino acid sequence encoded by SEQ ID NO:28
SEQ ID NO:30	Nucleotide sequence of coding region of SEQ ID NO:28
SEQ ID NO:31	Amino acid sequence encoded by SEQ ID NO:30
SEQ ID NO:32	Amino acid sequence of Group 10 allergen from <i>Dermatophagoides farinae</i> (Df10)
SEQ ID NO:33	Amino acid sequence of Group 10 allergen from <i>Lepidoglyphus destructor</i> (Lp10)

SEQUENCE IDENTIFIER	DESCRIPTION
SEQ ID NO:34	Amino acid sequence of Group10 allergen from <i>Dermatophagoides pteronyssinus</i> (Dp10)
SEQ ID NO:35	Amino acid sequence of Group 10 allergen from <i>Dermatophagoides pteronyssinus</i> (Dp10)
SEQ ID NO:36	Bt5 nucleotide sequence (ref 17)
SEQ ID NO:37	Bt5 amino acid sequence (ref 17)
SEQ ID NO:38	Bt5 polymorphic genomic sequence no. 1
SEQ ID NO:39	Bt5 polymorphic genomic sequence no. 2
SEQ ID NO:40	Bt5 polymorphic genomic sequence no. 3
SEQ ID NO:41	Bt5 polymorphic genomic sequence no. 4
SEQ ID NO:42	Bt5 polymorphic genomic sequence no. 5
SEQ ID NO:43	Bt5 polymorphic genomic sequence no. 6
SEQ ID NO:44	Bt5 polymorphic genomic sequence no. 7
SEQ ID NO:45	Bt5 polymorphic genomic sequence no. 8
SEQ ID NO:46	Bt5 polymorphic genomic sequence no. 9
SEQ ID NO:47	Bt5 polymorphic genomic sequence no. 10
SEQ ID NO:48	Bt5 polymorphic genomic sequence no. 11
SEQ ID NO:49	Bt5 polymorphic amino acid sequence nos. 1-3 and 7-10
SEQ ID NO:50	Bt5 polymorphic amino acid sequence no. 4
SEQ ID NO:51	Bt5 polymorphic amino acid sequence no. 5
SEQ ID NO:52	Bt5 polymorphic amino acid sequence no. 6
SEQ ID NO:53	Bt5 polymorphic amino acid sequence no. 11
SEQ ID NO:54	Bt5 leader sequence (ref 17)
SEQ ID NO:55	Bt5 cDNA variant no. 1(13)
SEQ ID NO:56	Bt5 cDNA variant no. 2(7)
SEQ ID NO:57	Bt5 cDNA variant no. 3(2)
SEQ ID NO:58	Bt5 cDNA variant no. 4(15)
SEQ ID NO:59	Bt5 cDNA variant no. 5(1)
SEQ ID NO:60	Bt5 cDNA variant no. 6(11)
SEQ ID NO:61	Bt5 cDNA variant no. 7(4)

SEQUENCE IDENTIFIER	DESCRIPTION
SEQ ID NO:62	Bt5 cDNA variant no. 8(12)
SEQ ID NO:63	Bt5 cDNA variant no. 9(24)
SEQ ID NO:64	Bt5 cDNA variant no. 10(9)
SEQ ID NO:65	Bt5 cDNA variant no. 11(3)
SEQ ID NO:66	Bt5 cDNA variant no. 12(5)
SEQ ID NO:67	Bt5 cDNA variant no. 13(10)
SEQ ID NO:68	Bt5 polymorphic amino acid variant no. 7
SEQ ID NO:69	Bt5 polymorphic amino acid variant no. 24
SEQ ID NO:70	Bt5 polymorphic amino acid variant no. 13
SEQ ID NO:71	Bt5 polymorphic amino acid variant no. 10
SEQ ID NO:72	DNA primer
SEQ ID NO:73	DNA primer
SEQ ID NO:74	Nucleotide sequence of clone encoding BtA2
SEQ ID NO:75	Amino acid sequence encoded by SEQ ID NO:74
SEQ ID NO:76	Primer sequence
SEQ ID NO:77	Primer sequence
SEQ ID NO:78	Primer sequence
SEQ ID NO:79	Primer sequence

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TABLE 3

Variant	Position	Residue	Hyrdophobic*	Charge	Chemical
Genomic					
SEQ ID NO:36,38-41 & 43-47	4	Lys	Phi	positive	basic
SEQ ID NO:42	4	Glu	Phi	negative	acidic
SEQ ID NO:36, 38-42 & 44-47	12	Asn	Phi	neutral	amide
SEQ ID NO:43	12	Asp	Phi	negative	acidic
SEQ ID NO:36, 38-40 & 42-10	17	Leu	Pho	neutral	aliphatic
SEQ ID NO:41	17	Trp	Pho	neutral	aromatic
SEQ ID NO:36, 38-41 & 43-47	33	Leu	Pho	neutral	aliphatic
SEQ ID NO:43	33	Ile	Pho	neutral	aliphatic
SEQ ID NO:36, 38-42 & 44-47	34	Leu	Pho	neutral	aliphatic
SEQ ID NO:43	34	His	Phi	positive	basic
SEQ ID NO:36, 38-42 & 44-47	117	Gln	Phi	neutral	aliphatic
SEQ ID NO:43	117	Lys	Phi	positive	basic
SEQ ID NO:36, 38-47	2	Glu	Phi	negative	acidic
SEQ ID NO:48	2	Gln	Phi	neutral	aliphatic
cDNA					
SEQ ID NO:36, 56 & 67	28	Lys	Phi	positive	basic
SEQ ID NO:63	28	Asn	Phi	neutral	amide
SEQ ID NO:36, 56 & 63	35	Tyr	Pho	neutral	aromatic
SEQ ID NO:67	35	Asp	Phi	negative	acidic
SEQ ID NO:36, 63 & 67	57	Arg	Phi	positive	basic
SEQ ID NO:56	57	Leu	Pho	neutral	aliphatic

* Phi: hydrophilic and Pho: hydrophobic

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing the culture apparatus for dust and storage mites.

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Figure 2 is a representation of the nucleotide sequence alignment of Clone 33 (*Bt11*) and Df 11.

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Figure 3 is a representation of the nucleotide sequence and the deduced amino acid sequence of the full-length *Bt11* cDNA gene after the hybridization of clone 33 and 5'-RACE product. The full-length *Bt11* cDNA gene is composed of 3111 nucleotide base pairs with a 2625 base pairs open reading frame from position 326-2950. An ATG (Met) sequence is found at position 326-328 while a TAA (stop) codon is found at position 2951-2953. The full-length sequence shows a 325 nucleotide base pairs of untranslated region on the 5' end and a 158 nucleotide base pairs of untranslated region on the 3' end. The gene codes for an 875-amino acid protein with a methionine on the N-terminus.

15

Figure 4 shows the amino acid sequence alignment of Bt11 with several paramyosin isolated from different invertebrates. CLUSTAL W Multiple Sequence Alignment program shows a significant homology of Bt11 to Df 11 and 3 other invertebrate paramyosins. Bt11 amino acid sequence shows 90% sequence homology with the *Dermatophagoides farinae* paramyosin (Df 11). Moreover, the Bt11 amino acid sequence exhibited 58% homology to the *Drosophila melanogaster* paramyosin, and 50% homology with *Onchocerca volvulus* paramyosin and 49% homology to *Caenorhabditis elegans* paramyosin. Results of Basic BLAST search using the BLASTP 2.0 program with the National Center for Biotechnology Information (NCBI) databank likewise revealed a significant homology of between Bt11 gene and other invertebrates paramyosins such as *Dirofilaria immitis*, *Brugia malayi*, *Schistosoma mansoni*, *Taenia soleum* and *Schistosoma japonicum*.

25

Figure 5 is a photographic representation showing SDS-PAGE (10% w/v) analysis of recombinant Bt11 protein expressed in *E. coli*. The marker with different molecular weights in kD is shown. Lanes 1 and 2 are two concentrations of Bt11 loaded.

- 5 **Figure 6** is a photographic representation showing autoradiography of human IgE dot blot assay showing the different intensity of IgE reactivity of the representative sera (1 to 4). Serum 1 is negative. Serum 2 is slightly positive and serum 3 is more reactive than serum 4 is positive. Each dot is loaded with the following: (a) Bt11 recombinant protein; (b) whole *B. tropicalis* mite extract; and (c) GST recombinant protein as a negative control. 2 µg per
10 dot is loaded for recombinant Bt11 and recombinant GST; 5 µg per dot is loaded for *B. tropicalis* extract, respectively.

Figure 7 is a representation of nucleotide sequence and deduced amino acid sequence of Clone 1.2.

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Figure 8 is a representation of the nucleotide and deduced amino acid sequence (in one-letter code) of the Bt10 allergen. Nucleotide sequence residues are numbered at the end of each row. The stop codon is marked with a dash.

- 20 **Figure 9** is a representation of the amino acid sequence alignment of group 10 from various mite species, i.e. *Lepidoglyphus destructor* (Lp 10), *Dermatophagoides farinae* (Df 10) and *Dermatophagoides pteronyssinus* (Dp 10). There are two Dp 10 amino acid sequences in the Genbank: Dp 10(A) [submitted by Smith, W] and Dp 10(S) [23].

- 25 **Figure 10** is a representation of IgE plaque immunoassay of Singapore and Taiwanese sera against Bt10-cDNA containing clone. Blots 1-13 were probed with mite-allergic individual sera, whereas blot 14 was non-atopic serum control. Sera for blots 1-9 and 10-13 are from Singapore and Taiwan, respectively.

Figure 11 is a photographic representation of SDS-PAGE showing purified recombinant fusion protein and thrombin-cleaved of GST-Bt10. Lane 1, protein molecular weight marker; lane 2, purified GST-Bt10; and lane 3, Bt10.

5 **Figure 12** is a photographic representation of IgE immunoblotting of two atopic sera (blot A and B) and one non-atopic serum (blot C) against, lane 1, molecular weight marker; lane 2, Bt crude extract; lane 3, GST; lane 4, GST-Bt10.

10 **Figure 13** shows the one-letter amino acid sequences deduced from the genomic sequences of the Bt5 variants as shown in Figure 21. Number on the top indicates the position of the amino residue.

15 **Figure 14** shows the nucleotide sequences of Bt5 cDNA variants (1-13). Number on the right indicates the nucleotide position. Sequences underlined are the start, stop codons and polyadenylated sites. Bt5 is the sequence published by Arruda *et al.* (15).

20 **Figure 15** is a representation of the nucleotide sequence of BtA2 (SEQ ID NO:74). The numbers indicate the nucleotide positions. The nucleotide sequence of the clone is 507 bp in length. This includes a linker sequence ggccagag, a 286 bp 3' untranslated region with a poly-A tail and a 218 bp probable truncated coding region for the recombinant protein with a stop codon (TAA) at nucleotide residue 219-221.

25 **Figure 16** is a representation of the nucleotide sequence and the deduced amino acid sequence of clone BtA2 (SEQ ID NO:74 and SEQ ID NO:75). The numbers indicate the nucleotide positions. The inferred amino acid sequence from nucleotides 9-218 indicated that this partial clone codes for a protein of 66 residues, with 8 cysteine residues in the molecules.

30 **Figure 17** is a photographic representation of the plaque IgE immunoassay showing the IgE reactivity of 19 mite allergic serum samples and 1 non-allergic serum sample tested with the recombinant protein BtA2. Eight serum samples showed strong positive, 7 serum

samples showed slightly positive and 4 serum samples showed negative IgE reactivity. Panel 20 represents the negative control. Panel numbers 2, 3, 4, 5, 6, 7, 8, 9, 11, 15, 16 and 17 are positive. Panel number 1, 14, 18 and 20 are slightly positive whereas panel number 10, 12, 13 and 19 are negative.

5

Figure 18 is a photographic representation of the SDS-PAGE (10% w/v) analysis of recombinant BtA2 protein expressed in *E. coli* (Gel A) and in *Pichia pastoris* (Gel B). The gels are stained with Coomassie brilliant blue R-250. The recombinant protein of clone BtA2 is approximately 7 kD. The GST-fusion protein of clone BtA2 is approximately 35
10 kD. From 1 litre culture of *E. coli*, 7-10 mg of GST-clone BtA2 fusion protein could be obtained. The maximum yield of secreted BtA2 protein in yeast was one the fourth day after methanol induction. The yield by then was approximately 2.5-3.5 mg for 1 litre of yeast culture. Gel A shows the molecular weight marker lane, two lanes with two different concentrations of GST-fusion BtA2 protein purified by glutathione affinity
15 chromatography and two lanes with different concentrations of elute (GST-BtA2 fusion protein which has been cleaved with thrombin and then purified by glutathione affinity chromatography. The elute is the 7.0 kD BtA2 recombinant protein). Gel B shows the molecular weight marker lane and the time kinetics of protein expression (day 1 to day 4) after the addition of 100% w/v methanol (to a final concentration of 0.5%) to the growth
20 medium of yeast culture.

Figure 19 is a photographic representation of the autoradiography of dot blot assay using the ECL detection reagents. The dot blot shows the IgE reactivity of the various sera (A-E). Each dot is loaded with the following: (1) 2 µg of BtA2 recombinant protein, obtained
25 after thrombin digestion of the GST-BtA2 fusion protein; (2) 2 µg of GST-BtA2 fusion protein; (3) 2 µg of GST-Bt5 fusion protein; (4) 2 µg of GST alone; and (5) 2 µg of *B. tropicalis* extract, respectively.

Figure 20 shows the sequence of the overlapping synthetic peptide and the peptide
30 variants. The peptides were designed to be 16-amino acid residues in length, overlapping by 13 amino acid residues. Peptides 35-67 are Bt5 peptides designed on the basis of

sequence published by Arruda *et al.* (15). Peptides 80-111 are the peptide variants of Bt5. These peptide variants contain naturally occurring mutations of Bt5 proteins.

5 **Figure 21** shows the nucleotide sequences of Bt5 genomic DNA obtained by PCR amplification from the environmental *Bt* mites. Intron sequences are indicated in lower cases. Sequences underlined are the start and stop codons. Sequences 1 to 5 and 6 to 11 representing Bt5 gene sequences obtained from Colombian and Singapore *Bt* mites, respectively. Reference to Bt5 sequences refers to the published cDNA sequence reported by Arruda *et al.* (15). Number on the right indicates the nucleotide position of the DNA
10 sequence.

Figure 22 shows the comparison of the deduced amino acid sequences for the Bt5 cDNA variants. Number on the right indicates the position of the amino acid residue.

15 **Figure 23** shows the sequence of the Bt5 peptide variants that contain at least one human T-cell epitope. These epitopes are located in the regions where sequence polymorphisms are found. (+)= weak T cell response; (++)= moderate T cell response; (+++)= strong T cell response; (-)= no T cell response. Polymorphic amino acid residues were denoted by single letter abbreviations for amino acids and the number indicated the residue position.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a novel protein from the domestic dust or storage mite or a derivative, homologue, analogue, chemical equivalent or mimetic thereof which protein is capable of inducing or facilitating the induction of an immune response to the domestic dust or storage mite. This protein is conveniently in isolated or purified form. The identification of this protein, and its encoding nucleic acid sequence, now permits, *inter alia*, the use of these molecules as therapeutic and diagnostic agents.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a dust mite or a derivative, homologue or mimetic of said protein allergen.

Reference to "mite" should be understood in its broadest sense and includes small arachnids of the order Acari. Preferred mites include mites from the family *Glycyphagidae*, such as the domestic mites *B. tropicalis* which are found in house dust and storage mites.

Accordingly, another aspect of the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is selected from Bt11, Bt10 and BtA2, each as herein defined, or polymorphic variants thereof and a polymorphic variant of Bt5 as herein defined.

Allergens from mites and in particular *B. tropicalis* are referred to herein as "Bt" allergens.

Reference to a protein "allergen" from a mite should be understood to mean that when introduced, in an effective amount, to sensitized individuals or individuals who are susceptible to sensitization, the protein will elicit, induce or otherwise facilitate an immune response. In this regard, the immune response may be a humoral and/or a cellular immune

response. In a preferred embodiment, the immune response comprises a humoral response component and, most particularly, an IgE response. In one particular embodiment, the allergen is tropomyosin or a homologue or derivative thereof. A "Bt" allergen includes a polymorphic variant of a Bt allergen. Examples of polymorphic variants include the
5 polymorphic variants of Bt5. The Bt5 allergen was disclosed by Arruda *et al.* (7). Examples of specific Bt5 polymorphic variants are those set forth in SEQ ID NO:38 to SEQ ID NO:53 (genomic sequences), SEQ ID NO:59 to SEQ ID NO:67 (cDNA sequences) and SEQ ID NO:68 to SEQ ID NO:71 (amino acid sequences).

10 According to this embodiment, there is provided an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is a polymorphic variant of Bt5 having an amino acid sequence as set forth in any one of SEQ ID NO:49 to SEQ ID NO:53 or
15 SEQ ID NO:68 to SEQ ID NO:71 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in any one of SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71.

More particularly, the present invention provides an isolated nucleic acid molecule or
20 derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is a polymorphic variant of Bt5 wherein said nucleotide sequence is substantially as set forth in any one of SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a derivative, homologue or mimetic
25 thereof or a nucleotide sequence having at least about 45% similarity to any one of SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a nucleotide sequence capable of hybridizing to any one of SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a complementary form thereof under low stringency conditions.

30 Reference to a "sensitized" individual should be understood as a reference to an individual who has been previously exposed to an allergen and upon subsequent exposure to the same

allergen mounts an immune response which utilizes memory B and/or T cells. An individual who is "susceptible" to the sensitization is reference to an individual who, upon exposure to the allergen for the first time, will mount a primary immune response to the allergen. The allergen may comprise one or more epitopic regions to which a humoral
5 immune response is directed. It may also, or alternatively, comprise one or more peptide regions to which a T cell response is directed upon processing and presentation of the protein by an antigen presenting cell.

It should also be understood that the allergen defined herein will not necessarily induce an
10 immune response in all individuals who are exposed to it. It should also be understood that even within a group of individuals who are responsive to the allergen, these individuals may be responsive only to a certain range of dosages of the allergen. In this regard, the principles of low and high dose tolerance are relevant wherein introduction of an immunogen, such as an allergen, at very high or very low doses sometimes induces
15 tolerance.

In one preferred embodiment, the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative,
20 homologue or mimetic of said protein allergen wherein said protein allergen is Bt11 having an amino acid sequence as set forth in any one of SEQ ID NO:2 to SEQ ID NO:14 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to an amino acid sequence set forth in in SEQ ID NO:2 to SEQ ID NO:14. This protein is referred to herein as "Bt11". The corresponding gene is "*Bt11*".

25 More particularly, the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is Bt11 wherein said nucleotide
30 sequence is as substantially set forth in SEQ ID NO:1 or SEQ ID NO: 2 or SEQ ID NO:13 or a derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:1

or SEQ ID NO:2 or SEQ ID NO:13 or a complementary form thereof under low stringency conditions.

5 In another preferred embodiment, the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is Bt10 having an amino acid sequence as substantially set forth in SEQ ID NO:29 or SEQ ID NO: 31 or a derivative, homologue or mimetic thereof having at least about 45% similarity to
10 amino acid sequences set forth in SEQ ID NO:29 or SEQ ID NO:31.

More particularly, the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of
15 said protein allergen, wherein said protein allergen is Bt10 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:28 or SEQ ID NO: 30 or a nucleotide sequence having at least 45% similarity to SEQ ID NO:28 or SEQ ID NO:30 or a nucleotide sequence capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or a complementary form thereof under low stringency conditions. This protein is referred to
20 herein as "Bt10". The corresponding gene is "*Bt10*".

In another preferred embodiment, the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative,
25 homologue or mimetic of said protein allergen, wherein said protein allergen is BtA2 having an amino acid sequence as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to an amino acid sequence set forth in SEQ ID NO:75.

30 More particularly, the present invention provides an isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a

sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen, wherein said protein allergen is BtA2 wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:74 or a derivative, homologue or mimetic thereof or a nucleotide sequence having at least about 45% similarity to SEQ ID NO:74 or a nucleotide sequence capable of hybridizing to SEQ ID NO:74. This protein is referred to herein as "BtA2". The corresponding gene is "*BtA2*".

The terms "protein" and "polypeptide" are used interchangeably herein to refer to a polymer of amino acid residues. For convenience, a "peptide" includes a "polypeptide".

10

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity.

20

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A

30

"comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.* (24). A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.* (25).

The terms "sequence similarity" and "sequence identity" as used herein refers to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation to sequence similarity.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions. Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may
5 be altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. In one particular embodiment, low stringency is regarded as low stringency at 42°C. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M
10 salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ [Marmur and Doty (26)]. However, the T_m of a duplex DNA decreases by 1°C with every increase
15 of 1% in the number of mismatch base pairs [Bonner and Laskey (27)]. Formamide is optional in these hybridization conditions. Accordingly, particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC buffer, 0.1% w/v SDS at a temperature in the
20 range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C.

Another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in SEQ ID NO:1, or a
25 derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:1 or its complementary form under low stringency conditions. This nucleotide sequence corresponds to "*Bt11*".

Preferably, the present invention contemplates a nucleic acid molecule encoding *Bt11* or
30 derivative thereof comprising a nucleotide sequence substantially as set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a derivative, homologue or mimetic thereof or

capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or their complementary forms under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:13 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:2 or SEQ ID NO:13.

10 An example of a derivative is a polymorphic variant defined of *Bt11* is by the nucleotide sequence set forth in SEQ ID NO:27 and with a corresponding amino acid sequence set forth in SEQ ID NO:27.

More particularly, the present invention contemplates a *Bt11*-encoding nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13.

15 Another aspect of the present invention contemplates a genomic nucleic acid molecule or derivative thereof capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or their complementary forms, or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C.

20 Reference herein to Bt11 and *Bt11* should be understood as a reference to all forms of Bt11 and *Bt11*, respectively, including, for example, any peptide and cDNA isoforms which arise from alternative splicing of *Bt11* mRNA or mutants or polymorphic variants of *Bt11* or Bt11. To the extent that it is not specified, reference herein to Bt11 and *Bt11* includes reference to derivatives, homologs, analogues, chemical equivalents and mimetics thereof.

25 In particular, the terms "Bt11" and "*Bt11*" include reference to polymorphic variants such as but not limited to the variant defined by the nucleotide sequence SEQ ID NO:27 and its corresponding amino acid sequence SEQ ID NO:27.

Another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in SEQ ID NO:28 or SEQ ID NO:30, or a derivative, homologue or mimetic thereof or capable of hybridizing to

SEQ ID NO:28 or SEQ ID NO:30 or complementary forms thereof under low stringency conditions. This nucleic acid molecule is referred to herein as "*Bt10*".

5 Preferably, the present invention contemplates a *Bt10*-encoding nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in SEQ ID NO:28 or SEQ ID NO:30 or a derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or their complementary forms under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in SEQ ID NO:28 or SEQ ID NO:30 or a sequence having at
10 least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:28 or SEQ ID NO:30 and wherein said amino acid sequence defines a polypeptide having tropomyosin activity.

15 More particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:28 or SEQ ID NO:30. This nucleic acid molecule is referred to herein as "*Bt10*". Reference herein to "*Bt10*" includes both a cDNA molecule and a genomic gene as well as any polymorphic variants thereof.

20 Another aspect of the present invention contemplates a genomic nucleic acid molecule encoding *Bt10* or derivative thereof capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or complementary forms thereof or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C. The genomic nucleic acid molecule preferably encodes a tropomyosin.

25 Reference herein to *Bt10* and *Bt10* should be understood as a reference to all forms of *Bt10* and *Bt10*, respectively, including, for example, any peptide and cDNA isoforms which arise from alternative splicing of *Bt10* mRNA or mutants or polymorphic variants of *Bt10* or *Bt10*. To the extent that it is not specified, reference herein to *Bt10* and *Bt10* includes
30 reference to derivatives, homologs, analogues, chemical equivalents and mimetics thereof.

Still another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in SEQ ID NO:74, or a derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:74 or its complementary form under low stringency conditions. This nucleic acid molecule corresponds to "*BtA2*".

More particularly, the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in SEQ ID NO:74 or a derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:74 or its complementary form under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in SEQ ID NO:75 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:75.

Even more particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:74.

Reference herein to *BtA2* and *BtA2* should be understood as a reference to all forms of *BtA2* and *BtA2*, respectively, including, for example, any peptide and cDNA isoforms which arise from alternative splicing of *BtA2* mRNA or mutants or polymorphic variants of *BtA2* or *BtA2*. To the extent that it is not specified, reference herein to *BtA2* and *BtA2* includes reference to derivatives, homologues, analogues, chemical equivalents and mimetics thereof.

The protein and/or gene is preferably from *B. tropicalis*. However, a protein and/or gene may also be isolated from other species of mite such as other mites from the family *Glycyphagidae*. The protein and/or gene may also be isolated from any non-mite species such as other members of the order Acari. The protein and/or gene may also be isolated from any mite or non-mite species other than those comprising the order Acari. Collectively, the allergens are referred to herein as "Bt" allergens. The corresponding

genes are referred to a "*Bt*". Accordingly, *Bt* includes *Bt11*, *Bt10*, *Bt5* and *BtA2* and *Bt* includes *Bt11*, *Bt10*, *Bt5* and *BtA2*.

- Without limiting the present invention to any one theory or mode of action, a *Bt* protein is proposed to be reactive with human IgE present in the serum of patients who are allergic to house dust or storage mites. It is thereby thought that a protein comprises at least one epitopic region to which a humoral immune response is directed in individuals who are sensitized to, or susceptible to, sensitization to mites. A *Bt* protein is defined by the amino acid sequence or its encoding nucleotide sequence as set forth above (see also Table 3).
- 10 The nucleic acid molecule encoding a *Bt* is preferably a sequence of deoxyribonucleic acids such as a cDNA sequence or a genomic sequence. A genomic sequence may also comprise exons and introns. A genomic sequence may also include a promoter region or other regulatory regions.
- 15 Another aspect of the present invention contemplates a genomic nucleic acid molecule or derivative thereof capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or their complemetnary forms or a derivative or homologue or mimetic thereof, under low stringency conditions.
- 20 Reference herein to *Bt* and *Bt* should be understood as a reference to all forms of *Bt* and *Bt*, respectively, including, for example, any peptide and cDNA isoforms which arise from alternative splicing of *Bt* mRNA or mutants or polymorphic variants of *Bt* or *Bt*. To the extent that it is not specified, reference herein to *Bt* and *Bt* includes reference to derivatives, homologs, analogues, chemical equivalents and mimetics thereof.
- 25 Derivatives include fragments, parts, portions, mutants, and mimetics from natural, synthetic or recombinant sources including fusion proteins. Parts or fragments include, for example, epitopic regions of the *Bt*. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or
- 30 carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid

residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. An example of substitutional amino acid variants are conservative amino acid substitutions. Conservative amino acid substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Additions to amino acid sequences including fusions with other peptides, polypeptides or proteins.

Homologs of the protein contemplated herein include, but are not limited to, proteins derived from different species.

Chemical and functional equivalents of *Bt* or Bt should be understood as molecules exhibiting any one or more of the functional activities of *Bt* or Bt and may be derived from any source such as being chemically synthesized or identified via screening processes such as natural product screening.

The derivatives of Bt include fragments having particular epitopes of parts of the entire Bt protein fused to peptides, polypeptides or other proteinaceous or non-proteinaceous molecules.

Analogues of Bt contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecules or their analogues.

Derivatives of nucleic acid sequences may similarly be derived from single or multiple nucleotide substitutions, deletions and/or additions including fusion with other nucleic acid

molecules. The derivatives of the nucleic acid molecules of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in co-suppression and fusion of nucleic acid molecules. Derivatives of nucleic acid sequences also include degenerate variants.

5

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; 10 trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

15

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

20

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitization, for example, to a corresponding amide.

25

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4- chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2- 25 chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

30

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide

or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

5 Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carboethoxylation with diethylpyrocarbonate.

10 Examples of incorporating unnatural amino acids and derivatives during protein synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid contemplated herein is shown in International Patent Application No. PCT/AU96/00668 [International Patent Publication No. WO 97/15663].

15

Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety.

20

The nucleic acid molecule of the present invention is preferably in isolated form or ligated to a vector, such as an expression vector. By "isolated" is meant a nucleic acid molecule having undergone at least one purification step and this is conveniently defined, for example, by a composition comprising at least about 10% subject nucleic acid molecule, preferably at least about 20%, more preferably at least about 30%, still more preferably at least about 40-50%, even still more preferably at least about 60-70%, yet even still more preferably 80-90% or greater of subject nucleic acid molecule relative to other components as determined by molecular weight, encoding activity, nucleotide sequence, base composition or other convenient means. The nucleic acid molecule of the present invention may also be considered, in a preferred embodiment, to be biologically pure.

30

As stated above, the term "protein" should be understood to encompass peptides, polypeptides and proteins. The protein may be glycosylated or unglycosylated and/or may contain a range of other molecules fused, linked, bound or otherwise associated to the protein such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins. Reference hereinafter to a "protein" includes a protein comprising a sequence of amino acids as well as a protein associated with other molecules such as amino acids, lipids, carbohydrates or other peptides, polypeptides or proteins.

10 In a particularly preferred embodiment, the nucleotide sequence corresponding to *Bt11* is a cDNA or genomic sequence comprising a sequence of nucleotides as set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a derivative thereof including a nucleotide sequence having at least about 45% similarity to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13.

15 In another particularly preferred embodiment, the nucleotide sequence corresponding to *Bt10* is a cDNA or genomic sequence comprising a sequence of nucleotides as set forth in SEQ ID NO:28 or SEQ ID NO:30 or a derivative thereof including a nucleotide sequence having at least about 45% similarity to SEQ ID NO:28 or SEQ ID NO:30.

20 In another particularly preferred embodiment, the nucleotide sequence corresponding to *BtA2* is a cDNA or genomic sequence comprising a sequence of nucleotides as set forth in SEQ ID NO:74 or a derivative thereof including a nucleotide sequence having at least about 45% similarity to SEQ ID NO:74.

25 A derivative of a nucleic acid molecule of the present invention also includes a nucleic acid molecule capable of hybridizing to a nucleotide sequence as set forth in one or more of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or SEQ ID NO:28 or SEQ ID NO:30 or any one of SEQ ID NO: 38 to SEQ ID NO: 48 or SEQ ID NO:55 to SEQ ID NO:67 or
30 SEQ ID NO:74 or complementary forms thereof under low stringency conditions. Preferably, low stringency is at 42°C.

The nucleic acid molecule may be ligated to an expression vector capable of expression in a prokaryotic cell (e.g. *E.coli*) or a eukaryotic cell (e.g. yeast cells, fungal cells, insect cells, mammalian cells or plant cells). The nucleic acid molecule may be ligated or fused
5 or otherwise associated with a nucleic acid molecule encoding another entity such as, for example, a signal peptide. It may also comprise additional nucleotide sequence information fused, linked or otherwise associated with it either at the 3' or 5' terminal portions or at both the 3' and 5' terminal portions. The nucleic acid molecule may also be part of a vector, such as an expression vector. The latter embodiment facilitates production of
10 recombinant forms of Bt which forms are encompassed by the present invention.

The present invention extends to the expression product of the nucleic acid molecules as hereinbefore defined.

15 One expression product is Bt11 having an amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:14 or is a derivative, homologue, analogue, chemical equivalent or mimetic thereof as defined above or is a derivative, homologue or mimetic having an amino acid sequence of at least about 45% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:14 or a derivative or
20 homologue or mimetic thereof.

Accordingly, another aspect of the present invention is directed to an isolated protein selected from the list consisting of:-

- 25 (i) a protein allergen from a mite or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- (ii) a protein allergen from *B. tropicalis* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- 30

(iii) a protein having an amino acid sequence substantially as set forth in SEQ ID NO:2 or SEQ ID NO:14 or a derivative, homologue or mimetic thereof or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:2 or SEQ ID NO:14 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;

(iv) a protein encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:14 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:2 or SEQ ID NO:14 or a derivative, homologue, analogue, chemical equivalent of said protein;

(v) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:14 or complementary forms thereof or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in SEQ ID NO:2 or SEQ ID NO:14 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:2 or SEQ ID NO:14;

(vi) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a homodimeric form; and

(vii) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a heterodimeric form.

Yet another expression product is Bt10 having tropomyosin activity and an amino acid sequence set forth in SEQ ID NO:29 or is a derivative, homologue, analogue, chemical equivalent or mimetic thereof as defined above or is a derivative, homologue or mimetic having an amino acid sequence of at least about 45% similarity to at least 10 contiguous

amino acids in the amino acid sequence as set forth in SEQ ID NO:29 or a derivative or homologue or mimetic thereof.

Accordingly, another aspect of the present invention is directed to an isolated protein
5 selected from the list consisting of:-

- (i) a protein allergen from a mite or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- 10 (ii) a protein allergen from *B. tropicalis* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- (iii) a protein having an amino acid sequence substantially as set forth in SEQ ID NO:29 or SEQ ID NO:31 or a derivative, homologue or mimetic thereof or a
15 sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:29 or SEQ ID NO:31 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;
- (iv) a protein encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:28 or SEQ ID NO:30 or a derivative or homologue thereof or a sequence
20 encoding an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:28 or SEQ ID NO:30 or a derivative, homologue, analogue, chemical equivalent of said protein;
- 25 (v) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in SEQ ID NO:28 or SEQ ID NO:30 or complementary forms thereof or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as
30 set forth in SEQ ID NO:28 or SEQ ID NO:30 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:28 or SEQ ID NO:30;

- (vi) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a homodimeric form; and
- 5 (vii) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a heterodimeric form.

Even still yet another expression product is BtA2 having an amino acid sequence set forth in SEQ ID NO:75 or is a derivative, homologue, analogue, chemical equivalent or mimetic
10 thereof as defined above or is a derivative, homologue or mimetic having an amino acid sequence of at least about 45% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in SEQ ID NO:75 or a derivative or homologue or mimetic thereof.

15 Accordingly, another aspect of the present invention is directed to an isolated protein selected from the list consisting of:-

- (i) a protein allergen from a mite or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- 20 (ii) a protein allergen from *B. tropicalis* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- (iii) a protein having an amino acid sequence substantially as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or a sequence having at least
25 about 45% similarity to at least 10 contiguous amino acids in SEQ ID NO:75 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;
- (iv) a protein encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:74 or a derivative or homologue thereof or a sequence encoding an amino acid
30 sequence having at least about 45% similarity to at least 10 contiguous amino acids

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in SEQ ID NO:75 or a derivative, homologue, analogue or chemical equivalent of said protein;

- 5 (v) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in SEQ ID NO:74 or a complementary form thereof or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in SEQ ID NO:75 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 45% similarity to at least 10 contiguous amino acids in SEQ ID
- 10 NO:75;
- (vi) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a homodimeric form; and
- 15 (vii) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a heterodimer form.

20 The present invention further extends to fragments of the Bt allergens comprising a linear or conformational epitope. For example, preferred fragments of Bt5 are set forth in Figure 20.

The Bt of the present invention may be in multimeric form meaning that two or more molecules are associated together. Where the same Bt molecules are associated together, the complex is a homomultimer. An example of a homomultimer is a homodimer. Where

25 at least one Bt is associated with at least one non-Bt molecule, and the complex is a heteromultimer such as a heterodimer. The heteromultimer may include, for example, another molecule in an amount capable of inducing tolerance to an allergen.

The ability to produce recombinant Bt permits the large scale production of Bt for

30 commercial use. The Bt may need to be produced as part of a large peptide, polypeptide or protein which may be used as is or may first need to be processed in order to remove the

- 40 -

extraneous proteinaceous sequences. Such processing includes digestion with proteases, peptidases and amidases or a range of chemical, electrochemical, sonic or mechanical disruption techniques.

- 5 Notwithstanding that the present invention encompasses recombinant proteins, chemically synthetic techniques also preferred in synthesis of Bt.

10 Bt according to the present invention is conveniently synthesized based on molecules isolated from *B. tropicalis*. Isolation of the *B. tropicalis* molecules may be accomplished by any suitable means such as by chromatographic separation, for example using CM-cellulose ion exchange chromatography followed by Sephadex (e.g. G-50 column) filtration. Many other techniques are available including HPLC, PAGE amongst others. Once purified, the Bt molecule can be partially sequenced and/or fragments produced induced directly as a source of Bt or at a template for amino acid synthesis.

15

Bt may be synthesized by solid phase synthesis using F-moc chemistry. Bt and fragments thereof may also be synthesized by alternative chemistries including, but not limited to, t-Boc chemistry or by classical methods of liquid phase peptide synthesis.

- 20 In accordance with the present invention, it is proposed that Bt is a mite derived protein allergen from mites which comprises at least one epitopic region to which an individual sensitized to mites, or an individual who is susceptible to sensitization to mites, may mount an immune response, such as a humoral IgE response. The identification of a novel mite allergen permits the generation of a range of molecules, such as therapeutic and
25 prophylactic molecules, for the treatment of conditions such as mite induced allergies. The identification of Bt also facilitates the generation of molecules for use as diagnostic agents.

Accordingly, the present invention provides a method of preventing, reducing or otherwise ameliorating a Bt hypersensitivity condition in a subject said method comprising
30 administering to said subject an effective amount of Bt or a derivative, homologue,

analogue, mimetic or chemical equivalent thereof for a time and under conditions sufficient to desensitize said individual.

The individual who is treated in accordance with the method of the present invention may be human or animal in need of therapeutic or prophylactic treatment and includes an individual who has become sensitized, or who is predisposed to becoming sensitized, to at least part of the Bt molecule such as an epitopic region of Bt. The Bt molecule, or part thereof, to which an individual becomes sensitized, may comprise part of any antigen such as, but not limited to, the dust or storage mite or a non-mite species.

Reference to "subject" should be understood as a reference to all animals including primates (e.g. humans, monkeys), livestock animals (e.g. sheep, cows, horses, donkeys, goats, pigs), laboratory tests animals (e.g. rats, guinea pigs, rabbits, hamsters), companion animals (e.g. dogs, cats), captive wild animals (e.g. emus, kangaroos, deer, foxes) avies (e.g. chickens, ducks, bantoms, pheasants, emus, ostriches), reptiles (e.g. lizards, snakes, frogs) and fish (e.g. trout, salmon).

Reference to "Bt hypersensitivity condition" should be understood as a reference to any of type I, II, III or IV hypersensitivity conditions directed to all or part of Bt. More particularly, the Bt hypersensitivity condition is a type I hypersensitivity condition. Examples of type I hypersensitivity conditions which may be treated in accordance with the method of the present invention include, but are not limited, atopy, immediate hypersensitivity, systemic anaphylaxis, allergic rhinitis (hayfever) or asthma (for example bronchial asthma).

Although the preferred method is to reduce or prevent the induction of an immune response to an antigen comprising all or part of Bt, it may be desirable to induce or up-regulate an immune response to such an antigen where, for example, the antigen is not innocuous. For example, where the antigen is a bacterium or parasite which comprises a all or part of a Bt region, it would be desirable to up-regulate an immune response to Bt.

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Accordingly, another aspect of the present invention relates to a method of modulating, in a subject, an immune response directed to Bt, said method comprising administering to said subject an effective amount of Bt or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to up-regulate,
5 down-regulate or otherwise modulate said immune response.

Reference to an "effective amount" should be understood as a reference to an amount of Bt or derivative, homologue, analogue, chemical equivalent or mimetic thereof necessary to at least partly achieve the desired outcome. For example, where it is sought to induce
10 tolerance to an antigen comprising a Bt portion, very low or very high concentrations of Bt may be administered to induce low or high dose tolerance, respectively. Alternatively, where it is sought to induce an immune response, doses of Bt which do not induce tolerance may be administered.

15 The present invention further extends to the use of Bt in the manufacture of a medicament for modulating an immune response.

Yet another aspect of the present invention provides an agent useful for modulating an immune response, said agent comprising Bt as hereinbefore defined.

20

Preferably, said modulation is down regulation of the immune response.

In accordance with these methods, more than one type of protein or peptide may be administered. For example, where the Bt is administered for the purpose of inducing
25 tolerance, the Bt may be co-administered with other known tolerance inducing compounds or molecules. Alternatively, where the Bt is administered to up-regulate the immune response, the Bt may be administered with an adjuvant. By "co-administered" is meant simultaneous administration in the same formulation or in two different formulations *via* the same or different routes or sequential administration by the same or different routes. By
30 "sequential administration" is meant a time difference of from seconds, minutes, hours or

days between the administration of the two or more types of molecules. The Bt and other compound or molecule may be administered in any order.

Routes of administration include but are not limited to intravenously, intraperitoneal, subcutaneously, intracranial, intradermal, intramuscular, intraocular, intrathecal, intracerebrally, intranasally, infusion, orally, rectally, *via iv drip*, patch and implant. Intravenous routes are particularly preferred. Administration may also be *via aerosol* or inhalation.

Another aspect of the present invention provides a composition for use in modulating an immune response comprising Bt as hereinbefore defined and one or more pharmaceutically acceptable carriers and/or diluents. The composition may also comprise two different types of molecules such as Bt and another compound or molecule with which it is co-administered.

Compositions suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. They must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients

enumerated above, as required, followed by, for example, filter sterilization or sterilization by other appropriate means. Dispersions are also contemplated and these may be prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those
5 enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, a preferred method of preparation includes vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution.

- 10 When the active ingredients are suitably protected, they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions,
15 syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or
20 preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 ng and 2000 mg of active compound.

- The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as
25 dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be
30 present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or

elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may
5 be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

10 Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is
15 contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to
20 physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the
25 particular therapeutic effect to be achieved and (b) the limitations inherent in the art of compounding such an active material.

Effective amounts of protein contemplated by the present invention will vary depending on the severity of the pain and the health and age of the recipient. In general terms, effective
30 amounts may vary from 0.01 ng/kg body weight to about 100 mg/kg body weight.

Alternative amounts include for about 0.1 ng/kg body weight about 100 mg/kg body weight or from 1.0 ng/kg body weight to about 80 mg/kg body weight.

5 The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of expressing Bt11 or derivative, homologue or mimetic thereof.

10 In particular, the composition comprises an isolated nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic or said protein allergen.

15 Preferably, the nucleotide sequence encodes the amino acid sequence of Bt11 substantially as set forth in SEQ ID NO:2 or SEQ ID NO:14 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in SEQ ID NO:2 or SEQ ID NO:14.

20 Even more preferably, the nucleotide sequence comprises the *Bt11* nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13, or a derivative, homologue or mimetic thereof, or capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 under low stringency conditions.

25 Preferably, the nucleotide sequence encodes the amino acid sequence of Bt10 substantially as set forth in SEQ ID NO:29 or SEQ ID NO:31 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in SEQ ID NO:29 or SEQ ID NO:31 and which corresponds to a polypeptide having tropomyosin activity.

30 Even more preferably, the nucleotide sequence comprises the *Bt10* nucleotide sequence of SEQ ID NO:28 or SEQ ID NO:30 or a derivative, homologue or mimetic thereof, or capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 under low stringency conditions.

Preferably, the nucleotide sequence encodes the amino acid sequence of BtA2 substantially as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in SEQ ID
5 NO:75.

Even more preferably, the nucleotide sequence comprises the *BtA2* nucleotide sequence of SEQ ID NO:74, or a derivative, homologue or mimetic thereof, or capable of hybridizing to SEQ ID NO:74 under low stringency conditions.
10

The present invention further contemplates the introduction of naked DNA as well as the viral or bacterial delivery of nucleic acid molecules.

Still another aspect of the present invention contemplates a method for the prophylactic
15 treatment of an allergic condition comprising the step of administering to an individual nucleic acid molecule comprising a gene encoding Bt or a derivative or homologue thereof whereby airway hyper-reactivity or airway inflammation is prevented.

Preferably, the allergic condition is allergic asthma, atopic dermatitis and/or rhinitis.
20

More preferably, the nucleic acid molecule is in the form of an eukaryotic expression vector. The eukaryotic expression vector may be selected from the group consisting of vectors with CMV promoter, RSV promoter and SV40 promoter and is preferably pCMV. The allergen may include any environmental antigen which can induce allergic reaction in
25 human such as mite allergens, glutathione S-transferase, pollen, animal dander, house dust* and peanut and the like.

The preferred allergic diseases include, for example, allergic asthma, allergic rhinitis, atopic dermatitis and anaphylaxis.
30

Preferably, the eukaryotic expression vector is administered in a pharmaceutical composition comprising a carrier selected from the group consisting of normal saline and a liposome. The pharmaceutical compositions of the invention are preferably administered by intramuscular injection, intranasal delivery or intratracheal delivery. The
5 pharmaceutically acceptable carrier may be conventional carriers useful for intramuscular injection, intranasal delivery or intratracheal delivery known in the art. For example, a physiologically acceptable buffer solution, normal saline, gold bead or liposome may be used.

10 Depending on the characteristic and progression of the disease to be prevented or treated in the individual and other factors such as age and physical conditions of the patient, the dosage of the recombinant plasmids ranges from about 0.01 to about 1.0 mg/kg body weight for a patient treated in accordance with the present invention.

15 Preferably, the individual is a human.

In relation to allergen gene transfer, this is predicated on DNA-based immunization which induces a biased Th1 immune response. This offers a strategy for modulating Th2 associated responses. In a preferred embodiment, allergen gene transfer immunization is by
20 the intramuscular injection of a plasmid DNA encoding a house dust or storage mite allergen (28).

Yet another aspect of the present invention is directed to antibodies to Bt and their derivatives, homologues, analogues, mimetics and chemical equivalents thereof. Such
25 antibodies may be monoclonal or polyclonal.

In the case of small peptides, these may first need to be associated with a carrier molecule. The antibodies of the present invention are particularly useful as therapeutic or diagnostic agents. For example, specific antibodies can be used to screen for Bt immunoassays or
30 used as antagonists to inhibit Bt activity under certain circumstances such as where temporary hypersensitivity inhibition only is required. Techniques for such immunoassays

are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of Bt levels may be important for monitoring certain therapeutic protocols.

Antibodies to the Bt (or its derivatives, homologues, analogues or mimetics) of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

- 10 As stated above, specific antibodies can be used to screen for the Bt. The latter would be important, for example, as a means for screening for levels of Bt in a biological fluid or purifying Bt made by recombinant means from culture supernatant fluid.

- 15 It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of Bt.

- 20 Both polyclonal and monoclonal antibodies are obtainable by immunization with Bt and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of Bt or antigenic parts thereof, collecting serum from the animal and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are
25 utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

- 30 The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The

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preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

- 5 Even still another aspect of the present invention is directed to methods of diagnosing individuals who have become sensitized to antigens which comprise all or part of Bt.

Accordingly, another aspect of the present invention contemplates a method for detecting antibody directed to all or part of Bt in a biological sample from a subject, said method
10 comprising contacting said biological sample with Bt or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient for an antibody-protein complex to form, and then detecting said complex.

Detection of the presence of Bt (for example, in a dust sample) or antibody to Bt may be
15 accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to U.S. Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody
20 to a target, such as Bt.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention to detect Bt or antibody to Bt. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present
25 invention. Briefly, in a typical forward assay, an unlabelled antibody of Bt is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen (or Bt-antibody) complex, a second antibody specific to the complex molecules, labelled with a reporter molecule capable of producing a
30 detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed

away, and the presence of the antigen or antibody is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain an antibody to Bt including cell extract, culture supernatant tissue biopsy, serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture. However, the sample may also be a sample thought to comprise the Bt molecule, such as a sample of dust thought to comprise dust or storage mite.

In the typical forward sandwich assay, a first antibody having specificity for the protein or antigenic parts thereof is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to about 37°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules (such as Bt) and then exposing the immobilized target to a sample which is to be tested for the presence of antibody to Bt. A second labelled reporter antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, luciferase glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody-peptide complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

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Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by
5 emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both
10 very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

Further features of the present invention are more fully described in the following non-
15 limiting Examples.

EXAMPLE 1

Culturing and harvesting of B. tropicalis mites

Bt dust or storage mites were grown in the laboratory and the starter cultures were prepared by collecting mites from the house dust samples in Singapore. *Bt* were identified according to Bronswijk (18) and isolated under a stereomicroscope from the dust samples, which were used for setting-up a starter culture (Figure 1). Fine powdered tetramin fish feed was used as a culture medium for culturing *Bt*. The tetramin flakes were heated at 60°C for 3 hours before being grounded to kill pre-existing mites and insects. It was then sieved through a 125 µm sieve before use. In order to provide a maximum surface area, a thin layer of <125 µm particles were introduced into Erlenmeyer flasks together with a few flakes that served as shelters and breeding ground for mites. A starter culture from a small bottle was inoculated into a one-litre Erlenmeyer flask which was then covered by two layers of paper towels to allow ventilation, and sealed with masking tape around the opening of the flask to prevent contamination from the other mites and insects. Those cultures were grown under natural environmental conditions with a mean annual temperature of 30°C and a mean RH of 80%.

Harvesting of dust or storage mites was performed when the culture was approximately 4-weeks old. Culture was observed under stereomicroscope to determine the purity of the mite populations. The mite culture was separated through a series of 500 µm and 125 µm sieves, by using a mechanical sieve shaker, where vibration was applied for about 20 minutes. Mites with sizes greater than 120 µm were transferred to a modified Tullgren, which was built up of 5 layers of gauze on a funnel that attached to a 15ml-Falcon tube (2097). A 60W bulb was applied from a distance of 15 cm from the culture medium for 4 hours. Most of the mites in the medium crawled through the gauze and down into the tube. Mites that remained in the funnel would then swept into the tube by a tiny soft brush after the gauze was removed. The purified mites were stored -80°C until use. Mites and the powered medium particles that were finer than 125 µm collected in a receiving pan placed at the bottom of the sieves were used for further subculturing.

"Examples 2 to 13 relate to Bt11"

EXAMPLE 2

Screening of Bt cDNA Library

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B. tropicalis cDNA library (Uni-ZAP XR Library) was screened by colony/plaque hybridization using the DIG system filter hybridization (Boehringer Mannheim). Digoxigenin (DIG)-labelled fragment 2 (f2) and fragment 4 (f4) of Df11 with nucleotides lengths of 431 and 604 bp, respectively, were used as molecular probes in the screening process. Phages were plated at 5-10,000 plaque forming units (pfu's) per 145 mm NZY agar petri dish. Pre-cooled plaques with a diameter of 1-2 mm after overnight incubation at 37°C were transferred to Hybond-N+ Nylon transfer membranes (Amersham) by colony/plaque lift method. Membranes were soaked in denaturation solution (0.5 N NaOH and 1.5 NaCl) for 5 minutes; followed by neutralization solution (1.0 M Tris-HCl and 1.5 M NaCl, pH 7.5) for 15 minutes; and in 2X SSC solution (0.3 mM NaCl and 30 mM sodium citrate, pH 7.0). Transferred DNA was crosslinked into the membrane by using a UV Stratalinker 1800 (Stratagene) at 1,200 µJoules x 100. Hybridization with the labelled probes was done overnight at 42.5°C in a hybridization chamber (Maxi 14 Hybaid™). Membranes were washed twice for 10 minutes each with 2X SSC (with 0.1% w/v SDS) at room temperature followed by two more washes at 15 minutes each with 0.5% SSC (with 0.1% w/v SDS) at 65°C. Unspecific sites were blocked with 10% blocking solution (Boeringer Mannheim) for 1 hour prior to incubation with anti-DIG antibody (1:10,000) for 30 minutes. After 4 times of washing with wash buffer (0.1 M Maleic acid, 0.15 M NaCl, 0.3% v/v Tween 20) for 15 minutes each, membranes were incubated for 2 minutes in detection buffer (0.1 Tris-HCl, 0.1 NaCl). Membranes were finally incubated in CSPD solution (1:100) for 5 minutes. Membranes placed in saran wrap were exposed to BioMax film (Kodak) for 15 minutes and developed in a dark room. Positive pfu's were picked from the agar plate using a cut 200 µl pipet tip. Clones were incubated at least for 24 hours in SM medium in a rotator drive. Clones were purified further by secondary and tertiary screening.

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EXAMPLE 3***Transformation of Positive Phage Clones***

Purified clones were transformed into *E. coli* SOLR strain using the single-clone excision
5 protocol described in the Uni-ZAP XR Library Instruction Manual (Stratagene). Briefly, a
mixture of 200 µl of *E. coli* XL1-Blue (O.D.=1.0), 30 µl of positive phage stock clone
(1X) and 1 µl of ExAssist helper phage ($>1 \times 10^6$ pfu/µl) was incubated at 37°C for 15
minutes. After the addition of 3 ml of LB broth, the reaction mixture was incubated for 3
hours at 37°C in a shaker (250 rpm). The cells were then heated at 70°C for 20 minutes in
10 a water bath followed by centrifugation at 3,500 rpm for 15 minutes. Two microliters of
the supernatant was added to 150 µl of overnight culture of *E. coli* SOLR strain (O.D.=1.0).
The reaction culture was incubated for 15 minutes at 37°C before plating 100 µl of the cell
mixture on LB-ampicillin agar plates. Plates were incubated overnight at 37°C.
Transformed clones were subcultured overnight in LB-ampicillin medium. Glycerol stock
15 of the different clones were stored at -80°C for future use.

EXAMPLE 4***DNA Sequencing***

20 Phage DNA of positive clones isolated and purified by using the Wizard Plus SV
minipreps DNA purification system (Promega) were determined and analyzed by DNA
sequencing. 50 to 80 ng of the amplified DNA was added to a 20 µl reaction mixture
containing 10 pmol of either T3 primer (5'AATTAACCCTCACTAAAGGG3') (SEQ ID
NO:19) or T7 primer (3'CGGGATATCACTC-AGCATAATG5') (SEQ ID NO:20) and 8
25 µl of Big Dye terminator RR mix (PE Applied Biosystems). Amplification was performed
by 25 cycles using the PTC-100 [Trademark] Programmable Thermal Controller (MJ
Research Inc., USA). Each cycle consists of 96°C for 30 seconds, 50°C for 15 seconds and
60°C for 4 minutes. DNA sequencing was performed using the ABI 377 DNA Sequencer
(Applied Biosystems).

EXAMPLE 5***Sequence Analysis***

The Bt11 nucleotide sequence was compared and aligned with the Df 11 nucleotide sequence using the CLUSTAL W version 1.6 multiple sequence alignment software. The reverse and complement sequences were performed using the GCG sequence analysis package. Likewise, the deduced nucleotide sequence was submitted to the databases of the National Center for Biotechnology Information (NCBI), using the BLAST network server for nucleotide and amino acid homology searches.

EXAMPLE 6***Human IgE Reactivity of Bt11 clone***

An initial experiment by plaque immunoassay to determine the frequency of human IgE reactivity of Bt11 clone 2 was carried out with 23 sera from donors with positive skin prick test to *B. tropicalis* extract. Prior to the experiment, human sera were adsorbed overnight at 4°C with XL1-Blue *E. coli* lysate. t 11 clone 2 was plated at approximately 1,000 plaque-forming units (pfu's) per 145 mm NZY agar plate. Plaques were transferred to nitrocellulose membrane [Hyband-C Extra membrane that was saturated with isopropyl- β -D-thiogalactopyranoside (IPTG)] by plaque lift method. After blocking the membrane with 5% solution of non-fat milk powder for 1 hour, it was incubated overnight with human sera (in 1% non-fat milk) at 4°C, followed by one hour incubation with alkaline phosphatase conjugated monoclonal anti-human IgE (1:10,000 dilution) at room temperature. Three times washing with PBS containing 0.05% v/v Tween 20 (PBST) was done in between the incubation steps. Membranes blotted on paper towels were finally incubated for 15-30 minutes in a developing solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Sigma) and nitro blue tetrazolium (NBT, Sigma) in 10 ml of AP buffer (1X). An intense bluish purple colour reaction indicated a positive reaction.

EXAMPLE 7

5'-Rapid Amplification of cDNA Ends (5'-RACE)

The 5' end of Bt11 cDNA clone was isolated using the 5'-Rapid Amplification of cDNA Ends (5'-RACE) following the protocol described in the SMART [Trademark] RACE cDNA Amplification Kit User Manual, protocol number PT3269-1 version PR93377 (Clontech Laboratories, Inc.) with slight modifications. Briefly, first-strand cDNA synthesis was performed using 1 µg of total RNA isolated from *B. tropicalis* culture. 5'-RACE Ready cDNA reaction mixture composed of 1 µl total RNA sample (1 µg), 1 µl 5'-cDNA Synthesis (CDS) primer, 1 µl SMART II oligo, and 2 ul RNase-free water was incubated at 70°C for 2 minutes followed by cooling on ice for 2 minutes. The 5 µl-reaction mixture was then mixed with 2 µl 5 x first-strand buffer, 1 µl 20 mM DTT, 1 µl 10 mM dNTP mix and 1 µl Superscript II (200 units/µl, Gibco BRL, USA). The reaction mixture was then incubated at 42°C for 90 minutes in an air incubator prior to dilution with 100 µl of Tricine-EDTA Buffer. The diluted reaction product was then heated at 72°C for 7 minutes. 5'-Rapid Amplification of cDNA Ends (5'-RACE) was performed using a 50 µl PCR reaction mixture composed of 2.5 µl 5'-RACE-Ready cDNA, 5 µl of 10 x Universal Primer Mix (UPM), 1 µl of 10 µM Gene Specific Primer 1 (GSP1) and 41.5 µl of Master Mix (34.5 µl PCR grade water, 5 µl 10X Advantage 2 PCR Buffer, 1 µl 10 mM dNTP Mix, and 1 µl 50X Advantage 2 Polymerase Mix). Positive and negative controls were prepared according to protocol. Gene-Specific Primer 1 (GSP1), 5'-CTTCATCCTCCAATCGGTGT3' (SEQ ID NO:21), and Gene-Specific Primer 2 (GSP2), 5'-GGTCGCTCAGAAGACGGTAC3' (SEQ ID NO:22), were designed from Bt11-clone 33 with a T_m of 60°C and 64°C, respectively. Amplification was performed by 25 cycles using the PTC-100 [Trademark] Programmable Thermal Controller (MJ Research Inc, USA). Each cycle consists of 94°C for 5 seconds, 65°C for 10 seconds and 72°C for 3 minutes. 5' RACE products were initially analyzed by agarose gel electrophoresis using 1% w/v agarose/EtBr gel. Positive RACE products were amplified using the same parameters mentioned above and was resolved using agarose gel electrophoresis followed by purification using the GenElute Spin Columns (Supelco 56500, USA). DNA samples were then precipitated by phenol/chloroform extraction.

Purified 5' RACE products were finally analyzed by DNA sequencing using the ABI 377 DNA Sequencer (Applied Biosystems).

EXAMPLE 8

5 *Hybridization of Bt11-clone 33 and 5'-RACE product*

The full-length Bt11 cDNA gene was generated by hybridizing a portion of the Bt11-clone 33 with the 5'-RACE product. The portion of Bt11-clone 33 used was an amplified segment of Bt11-clone 33 sequence by PCR using GSP1 primer and T7 primer in a 25- μ l
10 reaction consisting of 1 μ l of clone 33 plasmid DNA (100 ng), 1 μ l of GSP1 primer, 1 μ l T7 primer, 0.5 μ l of 10mM dNTPs, 2.5 μ l of 10X buffer, 0.375 μ l of enzyme (Expand High Fidelity PCR System; Boehringer Mannheim, USA) and 18.625 μ l of sterile water. Amplification was performed using the temperature profile: 94°C for 2 minutes followed by 9 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 30 seconds and
15 extension at 72°C for 1 minute with a touchdown profile of 19 cycles at 94°C for 15 seconds, 55°C for 30 seconds and 72°C for 1 minute with an increase of 20 seconds per cycle prior to a final extension at 72°C for 7 minutes. The amplified sample was purified as described for RACE product (F). Hybridization of the 2 strands was performed by PCR using 100 ng each of the purified Bt11-clone 33 segment cDNA and 5'-RACE product
20 DNA. A PCR reaction mixture of 25 μ l (composition as mentioned in F) was prepared using the Nested Universal Primer (NUP), 5'AAGCAGTGGT-AACAACGCAGAGT3' (SEQ ID NO:23), and the T7 primer, 3'CGGGATATCACTCAGC-ATAATG5' (SEQ ID NO:24). The DNA strands were denatured at 94°C for 30 seconds followed by annealing at 72°C for 5 minutes. Amplification was performed by 30 cycles using the PTC-100
25 [Trademark] Programmable Thermal Controller (MJ Research Inc., USA). Each cycle consists of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 3 minutes. PCR products were initially analyzed by agarose gel electrophoresis using 1% w/v agarose/EtBr gel. The PCR products were purified as described earlier and was finally analyzed by DNA sequencing using ABI 377 DNA Sequencer (Applied Biosystems).

EXAMPLE 9***Cloning of Full-Length Bt11 cDNA Gene***

The full-length Bt11 cDNA gene was ligated into pCR2.1-TOPO cloning vector and was
5 subsequently transformed into TOP10 One Shot [Trademark] competent cells using the
TOPO™ TA Cloning version E (Invitrogen). In order to do this, a single 3'-A overhang
was introduced to the full length Bt11 by incubating in a 100 µl reaction consisting of 1 µg
of purified DNA (20 µl), 2 µl 10 mM dATP, 10 µl of 10 x PCR buffer, 6 µl 25 mM MgCl₂,
1 µl of (5 u/µl) Taq Polymerase (Promega, USA) and 61 µl of sterile water at 72°C for 5
10 minutes.

The reaction was then purified using the GFX [Trademark] PCR DNA and Gel Band
Purification Kit (Pharmacia). The 100 µl reaction mix was added to 500 µl of Capture
Buffer. It was mixed thoroughly and transferred into one GFX column inserted into a
15 collection tube. This was centrifuged at 14,000 g for 30 seconds. The flow through was
discarded and the column was washed with 500 µl of Wash Buffer by repeated
centrifugation. The column was then transferred into a clean 1.5 ml microcentrifuge tube
and the DNA was eluted in 50 µl of elution buffer by centrifugation at 14,000 g for 1
minute after incubation at room temperature for 1 minute. Ligation was carried out with 2
20 µl of the purified full length Bt11 with 3'-A overhang added to 2 µl of dd water and 1 µl of
pCR2.1-TOPO vector. The ligation reaction was incubated for 5 minutes at room
temperature. Transformation was then performed by mixing 2 µl of the TOPO
[Trademark] Cloning reaction into a vial of TOP10 One Shot [Trademark] competent cells
which was previously treated with 2 µl of 0.5 M β-mercaptoethanol. After incubating the
25 One Shot [Trademark] transformation reaction for 30 minutes on ice, the cells were heat-
shocked at 42°C for 30 seconds in a water bath followed by 2-minute incubation on ice.
250 µl of SOC medium was then added to the reaction mixture prior to incubation at 37°C
for 30 minutes in a horizontal shaker. Cells were then plated onto selective LB-ampicillin
plates and was incubated overnight at 37°C. Transformed colonies, white colonies, were
30 subcultured overnight at 37°C on LB medium with ampicillin. Plasmid DNA purified by

Wizard Plus SV minipreps DNA purification system (Promega) were analyzed by DNA sequencing using the ABI 377 DNA Sequencer (Applied Biosystems).

EXAMPLE 10

Transformation of Full-Length Bt11 cDNA Gene

Cloned full-length Bt11 cDNA gene was purified by plasmid purification using the Wizard Plus SV minipreps DNA purification system (Promega). The full-length Bt11 cDNA gene was amplified by PCR using designed primers to create restriction enzyme sites for expression vector plasmid. Primer Bt11CS2f, 5'CGGGATCCATGGCGGCTCGATCAGCA3' (SEQ ID NO:25), was used to create a BamH1 restriction site on the 5' end while Primer Bt11R2, 3'CGCTCGAGTTAAAAGTTCGAGCTCTCTTG5' (SEQ ID NO:26), was used to create an Xho1 restriction site at the 3' end. Pfu polymerase was used in the amplification process. PCR was performed for 30 cycles consisting of 94°C for 15 seconds, 55°C for 30 seconds and 72°C for 1 minute using the PTC-100 [Trademark] Programmable Thermal Controller (MJ Research Inc). Gel-purified PCR product was digested with BamH1 and Xho 1. Restriction enzyme digested insert was purified by agarose gel electrophoresis followed by purification using GenElute spin columns (Supelco 56500) and phenol/chloroform extraction. Purified Bt11 insert was ligated overnight at 16°C into PGEX-4T-1 vector (Pharmacia Biotech) previously digested with BamH1 and Xho1. Ligated Bt11 insert and PGEX-4T-1 vector was transformed into *E. coli* BL21 strain by heat-shock method. A reaction mixture of 200 µl of thawed competent *E. coli* BL21 strain with 10 ng of ligation mixture was incubated on ice for 30 minutes. The reaction was heat-shocked at 42°C for 2 minutes and 30 seconds in a water bath followed by incubation on ice for 10 minutes. 800 µl of LB broth was then added to the reaction mixture which was incubated for one hour at 37°C. Cells were plated onto selective LB-ampicillin plates and was incubated overnight at 37°C. Transformed clones were subcultured in LB broth with ampicillin. Glycerol stock of the transformed clones were stored at -80°C for future use.

EXAMPLE 11***Protein Expression and Purification of Bt11***

The expression of recombinant Bt11 clone as a fusion protein with glutathione S-transferase (GST) was performed by isopropyl- β -D-thiogalactopyranoside (IPTG) induction. A 10X dilution of overnight culture of transformed clones was incubated approximately for 1 hour at 37°C in a shaker until the OD at 600 nm reaches 0.6. The expression was induced with 100 mM IPTG for 3 hours at 30°C. 300 μ l of the culture was taken before and after IPTG induction. Collected samples were pelleted and cells were resuspended in 25 μ l sample buffer. Denatured samples were analyzed by SDS-PAGE. Purification of the expressed protein was performed by affinity chromatography using the Glutathione Sepharose Column (Sigma). IPTG-induced bacterial pellet resuspended in tris-buffered saline (TBS) was sonicated using the Ultrasonic Processor XL (Heat Systems). Upon centrifugation at 17,000 g for 30 minutes, the supernatant was collected and loaded onto the Glutathione Sepharose Column and was eluted with 10 mM of Glutathione elution buffer as suggested by the GST Purification Module (Pharmacia Biotech). Purified protein samples were analyzed by SDS-PAGE and quantified by DC Protein Assay (BioRad).

EXAMPLE 12***Dot blot immunoassay***

The allergenicity of the recombinant Bt11 was determined *in vitro* by dot blot immunoassay using sera from mite sensitive patients. In brief, nitrocellulose membrane (Hybond C, Amersham Life Sciences) was cut to size, soaked in 1X PBS (pH 7.4) and place on top of 3 mm Whatmann paper before finally being clamped into a BioDot apparatus (Bio-Rad). Two μ g of purified Bt11 suspended in 200 μ l PBS was applied per dot and drawn through the nitrocellulose by suction with a vacuum pump. Five μ g per dot of crude Bt protein extract was used as positive control while 2 μ g of purified GST was used as negative control. All dots were done in duplicates. The nitrocellulose was then removed and blocked with 5% w/v non-fat milk powder in PBST (PBS with 0.05% v/v

Tween 20) for 1 hour at room temperature, followed by 6 washes with PBST for 5 minutes each. The nitrocellulose was then cut into various strips and labelled accordingly. The strips were incubated overnight at 4°C with 5X dilution of human sera in PBST. After thorough washing with PBST (6X for 5 minutes each), the membranes were incubated with biotinylated anti-human IgE (2 µg/ml) for 1 hour at room temperature followed by 6 washes with PBST for 5 minutes each. The membranes were then incubated with 5000X dilution of ExtrAvidin (peroxidase and alkaline phosphatase conjugated) for 1 hour followed by 6X wash with PBST. Positive reactions were detected using the ECL+PLUS (Amersham Pharmacia Biotech). Membranes were exposed to negative BioMax film (Kodak) and was developed according to the specifications of the manufacturer. These membranes were briefly washed with PBST and finally incubated in a developing solution containing BCIP and NBT in 10 ml of 1X AP buffer.

EXAMPLE 13

Identification and characterization of Bt11

Screening of approximately 6×10^4 plaque forming units (pfu's) from the Bt cDNA library resulted to 36 positive plaques using a combination of DIG-labeled Df 11 fragment 2 and fragment 4 as molecular probes. Four of these plaques, designated as clone 2, clone 8, clone 32 and clone 33 were purified to homogeneity by secondary and tertiary screening process. Nucleotide sequence analysis revealed that the four clones are identical with different degree of truncations on the 5' end. The nucleotide sequence of the 4 clones showed polyadenylation sequences. CLUSTAL W Multiple Alignment Program revealed that the 4 clones exhibited significant alignment to Df 11 nucleotide sequence.

Out of the four clones, clone 33 exhibited the longest size comprising of 2,598 bp with a polyadenylation sequence from position 2581 to 2598. Alignment of the nucleotide sequence of clone 33 with Df 11 nucleotide sequence revealed 89% sequence homology as shown in Figure 2. Clone 33 nucleotide sequence revealed the absence of any methionine sequence indicating therefore that it is truncated on the 5' end. Several batches of screening process failed to isolate the full-length Bt11 gene by colony/plaque hybridization. The 5'-

Rapid Amplification of cDNA Ends (5'-RACE) was used to fish out the full-length clone using designed gene specific primers (GSP's) based on the nucleotide sequence of the truncated clone 33.

- 5 Figure 3 shows the nucleotide sequence and the deduced amino acid sequence of the full-length Bt11 cDNA gene after the hybridization of clone 33 and 5'-RACE product. The full-length Bt11 cDNA gene is composed of 3111 nucleotide base pairs with a 2625 base pairs open reading frame from position 326-2950. An ATG (Met) sequence is found at position 326-328 while a TAA (stop) codon is found at position 2951-2953. The full-length sequence shows a 325 nucleotide base pairs of untranslated region on the 5' end and a 158 nucleotide base pairs of untranslated region on the 3' end. The gene codes for an 875-amino acid protein with a methionine on the N-terminus.
- 10

- Figure 4 shows the amino acid sequence alignment of Bt11 with several paramyosins isolated from different invertebrates. CLUSTAL W Multiple Sequence Alignment program shows a significant homology of Bt11 to Df 11 and 3 other invertebrate paramyosins. Bt11 amino acid sequence shows 90% sequence homology with the *Dermatophagoides farinae* paramyosin (Df 11). Moreover, the Bt11 amino acid sequence exhibited 58% homology to the *Drosophila melanogaster* paramyosin, 50% homology with *Onchocerca volvulus* paramyosin and 49% homology to *Caenorhabditis elegans* paramyosin. Results of Basic BLAST search using the BLASTP 2.0 program with the National Center for Biotechnology Information (NCBI) databank likewise revealed a significant homology of between Bt11 gene and other invertebrates paramyosins such as *Dirofilaria immitis*, *Brugia malayi*, *Schistosoma mansoni*, *Taenia soleum* and *Schistosoma japonicum*.
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Results of the human IgE plaque immunoassay using clone 2 (truncated clone) showed that out of 23 sera tested, 9 showed positive IgE reactivity. Table 4 summarizes the results of the IgE plaque immunoassay.

TABLE 4 Summary of the results of human IgE plaque immunoassay

	Positive	Negative	Total Sera Tested
Singapore sera	2	10	12
Taiwan sera	7	4	11

Dot blot immunoassay using the full-length Bt11 showed 68% positive reaction with human IgE from the Singapore sera tested (13/19) while only 30% positive reactions were observed from the Taiwan sera screened. All sera used are positive for IgE reactivity with the crude mite extract. Table 5 summarizes the result of the dot blot immunoassay. Figure 6 shows the representatives of the dot blot immunoassay.

10 **TABLE 5** Summary of the results of dot blot immunoassay

	Positive	Negative	Total Sera Tested
Singapore sera	13 (68%)	6	19
Taiwan sera	11 (30%)	26	37

A polymorphic variant of the Bt11 gene were isolated by Rapid Amplification of cDNA Ends (RACE) using mRNA extracted from mite cultures. The nucleotide and deduced amino acid sequences of the variant designated as Clone 1.2 is summarized in Figure 7.

"Examples 14 to 27 relate to Bt10"

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EXAMPLE 14*Immunization of mice*

Each mouse was immunized with 25 µg recombinant Dp 10 with the present of CFA (Complete Freund's Adjuvant). Mice were further challenged with Dp 10 and IFA

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(Incomplete Freund's Adjuvant) at day 14 and 21. ELISA was performed to determine the titer of IgG produced.

EXAMPLE 15

cDNA library screening

Screening of the *B. tropicalis* Uni-ZAP cDNA library was performed by plaque immunoassay methods, using mouse anti-Dp 10 polyclonal antibodies prepared as described in Example 14. Phages were plated at 10,000 plaque-forming units (pfu) per 135mm petri dish. Plaques were lifted onto IPTG (isopropyl- β -D-thiogalactopyranoside) saturated nitrocellulose filters (Amersham). The filters were blocked in PBS containing 0.05% v/v Tween 20 (PBST) and 5% w/v non-fat milk. After washing, the filters were incubated with preabsorbed mouse serum diluted 1:500 in blocking solution overnight at 4°C with continuous shaking. The filters were then vigorously washed thrice with PBST, followed by incubation with alkaline phosphatase conjugated anti-mouse IgG (Sigma) at room temperature for an hour. The filters were then washed as before and were subsequently developed with BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium). The positive plaques were picked and further screened for a purified clone.

EXAMPLE 16

DNA sequencing of Bt10 cDNA clone

The *Bt10*-containing phagemid was excised *in vivo* using f1 helper phage as described in the instruction manual of Zap-cDNA synthesis kit (Stratagene, La Jolla, CA). DNA was isolated and subjected to sequencing using ABI 377 DNA Sequencer (Applied Biosystems).

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EXAMPLE 17***Sequence analysis***

The sequences were aligned and homology analysis was performed using the BLAST
5 network service provided by National Center for Biotechnology Information (NCBI).

EXAMPLE 18***Frequency of IgE binding***

10 The frequency of IgE binding was determined by plaque immunoassay methods. A
purified *Bt10* clone was plated out densely and were lifted onto IPTG saturated
nitrocellulose filters (Amersham). Lifted filters were cut into sectors and probed with 13
mite-allergic individual sera, and 1 non-atopic serum as negative control. Bound IgE was
detected with anti-human IgE alkaline phosphatase conjugated (Sigma).

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EXAMPLE 19***Subcloning of Bt10 inserts into expression vector***

The *Bt10* cDNA insert was released and ligated to pGEX-4T1 vector with *Bam*HI and
20 *Eco*RI restriction enzyme sites, and transformed to BL21. The fusion protein was produced
by 1 mM IPTG induction and purified using glutathione Sepharose. Bt10 was then
subjected to cleaving by trombin. The IgE-immunoblotting was performed to determine
the allergenicity of the fusion protein.

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EXAMPLE 20***SDS-PAGE and IgE immunoblot assay***

Immunoblots were prepared by loading about 200 ng/well of GST-Bt10 and GST on a
7.5% w/v SDS-PAGE, followed by electro-blotting onto nitrocellulose membranes
30 (Hybond-C extra, Amersham Life Science, England). The allergenicity of Bt10 was
determined using IgE immunoblotting. Briefly, the blots were blocked in PBS containing

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0.05% v/v Tween 20 (PBST) and 5% w/v non-fat milk. After washing, each blot was incubated with 600 μ l of individual allergic serum diluted 1:5 in blocking solution overnight at 4°C with continuous shaking. The blots were then vigorously washed thrice with PBST, followed by incubating with 2 μ g/ml of biotin-anti human IgE (PharMingen, San Diego, CA) at room temperature for an hour. The blots were then washed as before and were subsequently incubated with ExtrAvidin conjugated with peroxidase (Sigma) (1:4000) for another hour at room temperature. After washing, the signals were developed with ECL-PLUS western blotting detection system reagent kit (Amersham Life Science, England) and autoradiography.

EXAMPLE 21

Skin prick tests

Skin prick tests were conducted according to the protocol previously described (5). Briefly, the skin of the forearm's volar was pricked with a disposable lancet with the presence of an allergen droplet. The prick was regarded positive when a wheal diameter is greater than 3X 3 mm after subtracting the negative control 30 minutes after the prick. Glycerol-buffer and 1 mg/ml of histamine were used as negative and positive controls, respectively. 25 μ g/ml of purified allergens were used in this study.

EXAMPLE 22

DNA sequence analysis

Two positive plaques were obtained after screening of approximately 6×10^4 pfu from the Bt cDNA library, designating clone 1 and 2. These clones were further purified to homogeneity and the inserts were sequenced. Nucleotide sequence analysis revealed that clone 2 is truncated at the 5' end. The complete nucleotide sequence was obtained in the other clone, consisting of 1014 base pair (Figure 9), including 6 bp long 5' non-translating region. The sequence has an ATG start codon at position 7 to 9, and a stop codon, TAA at position 859 to 861, which showed an open reading frame coding for a 284 amino acid molecule. The full length clone is designated "Bt10". The corresponding protein is referred

to as "Bt10". The nucleotide and corresponding amino acid sequence of *Bt10* are defined in SEQ ID NO:28 and SEQ ID NO:29, respectively.

EXAMPLE 23

Protein sequence analysis

Protein sequence homology search was performed by using the Advance BLAST search from NCBI. The search results shows a very significant homology of Bt10 to other invertebrates tropomyosin, especially from *Lepidoglyphus destructor*, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Bt10 showed 96% identity to published Der f 10 and Lep d 10. Sequence alignment also showed Bt10 shared 95% of identity to both of Der p 10 sequence. Figure 8 shows the alignments of the deduced amino acid of tropomyosin from various species of mites mentioned before. The alignments were performed using the CLUSTAL W Multiple Sequence Alignment package.

EXAMPLE 24

IgE plaque immunoassay

Nine out of 13 mite-allergic individuals' sera used in this assay showed a positive result, which contributed to 69% of IgE reactivity. There is no IgE binding on the sector that incubated with non-atopic serum (Figure 10).

EXAMPLE 25

Expression and purification of GST-Bt10

Expression of recombinant fusion Bt10 clone gave a single band at around 60 kDa on a 7.5% w/v SDS-PAGE (Figure 11). Lane 3 of Figure 11 is Bt10 that was ran at around 35 kDa and being released by trombin digestion followed by glutathione agarose purification. The calculated molecular weight of Bt10 is 33.2 kDa, which is similar to Mag 44, as reported by Aki *et al.* (29).

EXAMPLE 26***IgE-binding study of recombinant fusion Bt10***

5 The ability of IgE-binding of recombinant fusion Bt10 was determined by Western Blotting (Figure 12). The results showed that the Bt10 fusion produced is able to bind IgE of mite-sensitized individual significantly (Lane 4, Figure 12). Individual B also reacted to degraded product of GST-Bt10, which ran at lower molecular weight, shown in lane 4, blot B, Figure 12. The IgE-binding band at around 35 kDa in Bt crude extract might be the corresponding protein that is present in nature (Lane 2, Figure 12). Non-fused GST was
10 loaded next to fusion Bt10 in order to show the IgE reactivity is specific to Bt10 but not GST (Lane 3, Figure 12). The two high molecular weight proteins was due to the non-specific binding as indicated by negative control (Lane 1, blot C, Figure 12).

EXAMPLE 27

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Skin Prick Test

The trombin-cleaved Bt10 was tested *in vivo* by the skin prick test. Thirteen out of 109 mite-sensitized volunteers showed positive reaction to Bt10, which contributed to approximately 12% of the volunteers tested. Fifty-four of the volunteers were also tested
20 for shrimp reactivity and 11 of them reacted positively. Three of these shrimp-positive individuals also have positive SPT to Bt10, which might be due to the cross-reactive allergen, tropomyosin (30).

"Examples 28 to 39 relate to BtA2"

25

EXAMPLE 28***Serum samples***

Sera from 4 adults show showed positive skin prick test reaction to *B. tropicalis* extract
30 were pooled and used to screen the *B. tropicalis* cDNA λ gt11 library by plaque IgE immunoassay.

EXAMPLE 29***Screening of λ gT11 expression library of *B. Tropicalis****

5 Library screening was performed by plaque IgE immunoassay. The pooled sera was adsorbed with lysate prepared from *E. coli* (Y1090) and recombinant *E. coli* containing GST-Bt5 overnight at 4°C on an orbital rotator before use. Phages were plated at 5-10,000 plaque-forming units (pfu) per 145 mm petri dish. Plaques were transferred to nitrocellulose filters (Amersham) that were saturated with isopropyl- β -D-
10 thiogalactopyranoside (PITG). After blocking the filters with 5% w/v solution of non-fat milk powder for 1 hour, they were then washed three times with PBS containing 0.05% v/v Tween-20 (PBST), followed by overnight incubation with sera (1:1 dilution in PBST with 1% w/v non-fat milk and 0.02% w/v sodium azide) at 4°C. These filters were again washed three times. They were then incubated with the monoclonal anti-human IgE that was
15 alkaline phosphatase conjugate (1:1,000 dilution) for an hour at room temperature followed by three washes in buffer. After the last wash, the filter is blotted on paper towels and then incubated in developing solution containing 5-bromo-4-chloro-3-indolyl phosphate (BCIP, Sigma) and nitro blue tetrazolium (NBT, Sigma), for half to an hour. An intense bluish purple colour reaction signal indicates a positive reaction. The clone that
20 reacts positively in the first round was subjected to several additional rounds of plating and screening to obtain a pure single plaque. The frequency of IgE reactivity of the clone was carried out using sera from the mite allergic patients. The frequency study was performed using the plaque IgE immunoassay as described above.

25

EXAMPLE 30***Human IgE reactivity of BtA2 clone of *B. tropicalis****

To study the frequency of IgE reactivity of clone BtA2 in the population, the following experiment was performed. 19 sera from candidates with positive skin prick test to *B.*
30 *tropicalis* extract and a negative control serum were taken. Individual serum from each candidate was adsorbed with lysate prepared from *E. coli* (Y1090) and recombinant *E. coli*

containing GST-B t5 (1:1 dilution) overnight at 4°C on an orbital rotator before use. BtA2
λgt 11 clone was plated at approximately 1,000 plaque-forming units (pfu) per 145mm
petri dish. Plaques were transferred to nitrocellulose filters (Amersham) that were saturated
with isopropyl-β-D-thiogalactopyranoside (IPTG). After blocking the filters with 5% w/v
5 solution of non-fat milk powder for 1 hour, they were then washed three times with PBS
containing 0.05% v/v Tween-20 (PBST), followed by overnight incubation with sera (with
1% w/v non-fat milk and 0.02% w/v sodium azide) at 4°C. These filters were again washed
three times. They were then incubated with the monoclonal anti-human IgE that was
alkaline phosphatase conjugate (1:1,000 dilution) for an hour at room temperature
10 followed by three washes in buffer. After the last wash the filter was blotted on paper
towels and then incubated in developing solution containing 5-bromo-4-chloro-3-indolyl
phosphate (BCIP, Sigma) and nitro blue tetrazolium (NBT, Sigma), for half to an hour. An
intense bluish purple colour reaction signal indicates a positive reaction (Figure 17).

EXAMPLE 31

Dot blot immunoassay

The pattern of reactivity of allergic sera against BtA2, GST-BtA2, GST-Bt5 and GST were
analyzed by dot blot. A total of 5 sera were used. These sera were taken from candidates
20 with positive skin prick test with *B. tropicalis* extract. Nitrocellulose was cut to size,
soaked in PBS (pH 7.4) and placed on 3 mm Whatmann paper before finally being
clamped into a BioDot apparatus (Bio-Rad Laboratories) Samples with 2 µg of BtA2,
GST-BtA2, GST-Bt5 and GST, and 5 µg of *B. tropicalis* extract were applied in a volume
of 200 µl and drawn through the nitrocellulose by suction with the help of a vacuum pump.
25 The nitrocellulose paper was then removed and blocked with 5% w/v non-fat milk powder
in PBST (PBS with 0.05% v/v Tween 20) for 1 hour at room temperature, followed by
three washes with PBST, 10 minutes each. The nitrocellulose paper was cut into various
strips and labelled accordingly. These strips were incubated with their respective sera
overnight at 4°C in a shaker. After which, these strips were washed three times, 15 minutes
30 each with PBST at room temperature; incubated with biotin anti-human IgE (2 µg/ml) for
1 hour at room temperature followed by another 3 washes of 15 minutes each; incubated

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with ExtrAvidin peroxidase and ExtrAvidin alkaline phosphatase for further 1 hour also at room temperature and a final 3 washes with PBST of 15 minutes each. The ECL+PLUS (Amersham Life Sciences) western blotting detection system reagent kit was used. Negative film (Kodak) was exposed to the nitrocellulose paper and developed according to the specifications of the manufacturer (Figure 19).

EXAMPLE 32

Isolation of DNA from λ gt11 cDNA clone

Phages from immuno-positive plaques were amplified by the liquid lysate method. DNA was isolated using the Magic Lambda Preps DNA Purification System kit (Promega) according to the manufacturer's instructions.

EXAMPLE 33

Polymerase chain reaction amplification

Approximately 40-60 ng of DNA was added in a 25 μ l reaction mixture containing 0.4 pmol of λ gt121 forward and reverse primers, 0.2 mM of dNTPs, 1U of Native pfu DNA polymerase, 2.5 μ l of 10X Native pfu DNA polymerase buffer (Stratagene). Forty cycles of amplification was performed using a GeneAmp PCR System 2400, Perkin Elmer. Each cycle consists of 95°C for 1 minute 30 seconds, 65°C for 1 minute 30 seconds and 72°C for 2 minutes. The PCR DNA was further purified after gel electrophoresis using QIAEII Gel Purification Kit (150), Qiagen.

EXAMPLE 34

DNA sequencing

50 to 80ng of the amplified DNA was added to a 20 μ l reaction mixture containing 0.16 pmol of either λ gt11 forward or reverse primer and 8 μ l of BigDye terminator RR mix (PE Applied Biosystems), following the detailed protocol of the manufacturer. 25 cycles of

amplification were performed in a Gene Amp PCR System 2400. Each cycle consists 96°C for 30 seconds, 50°C for 30 seconds and 60°C for 4 minutes.

EXAMPLE 35

Expression of BtA2 recombinant protein

The BtA2 was successfully cloned in the pGEX system for recombinant protein expression in *E. coli* (Pharmacia) as well as in *Pichia* vector (pPIC9) for expression as a secreted protein in *Pichia pastoris* (Invitrogene) following the protocols of the manufacturers.

EXAMPLE 36

Expression of recombinant BtA2 clone in E. coli

The BtA2 clone was amplified by PCR using the primers 5'-CGGGATCCGCTCTCGACTTTACCAGC-3' (*Bam*H1 site in italics) [SEQ ID NO: 76] and 5'-CGGAATTCTTAACCCCTGGAGGGCAG-3' (*Eco*R1 site in italics) [SEQ ID NO: 77], the PCR product was cut with *Bam*H1 and *Eco*R1. The purified clone BtA2 from *Bam*H1 and *Eco*R1 digest was ligated to pGEX-4T1 vector (Pharmacia Biotech) and transformed into *E. coli* BL21. Recombinant clones were confirmed by restriction analysis of a miniprep from transformed BL21. Bulk culture of recombinant was grown in Luria broth containing 100 µg/ml of ampicillin to log phase and then induced with 1 mM of isopropyl β-D-thiogalactopyranoside (IPTG). Cells were harvested 2 hours later and washed once with TBS, pH 7.4. Pelleted cells were thoroughly sonicated in TBS, pH 7.4 in the presence of 1 mM phenylmethylsulfonyl fluoride (PMSF), 20 µg/ml of DnaseI and 1% v/v Tween 20, and centrifuged. The supernatant was applied to glutathione sepharose (Sigma) column followed by elution with 10 mM reduced glutathione in 50 mM Tris, pH 8.0 as suggested by the GST purification Module (Pharmacia Biotech). The GST-BtA2 protein was cleaved with the enzyme thrombin (1 Unit thrombin per 40.D. of the fusion protein at 280 nm wavelength) in the presence of 1 mM of CaCl₂ with gently shaking at 4°C overnight. The reaction mixture was then passed through the glutathione sepharose column and the elute was the 7 kD recombinant protein BtA2 (Figure 18, Gel A). GST was

bound to the column and it could be eluted out by the addition of 2 ml of Glutathione Elution Buffer (10 mM reduced glutathione in 50 mM Tris, adjust the pH to 8.0) per ml of column matrix.

5

EXAMPLE 37

Expression of recombinant BtA2 clone in the yeast Pichia Pastoris

The BtA2 clone was amplified by PCR using the primers 5'-GAAAAGAGCTCTCGACTTTACCAGC-3' [SEQ ID NO: 78] and 5'-
10 CGGAATTCTTAACCCCTGGAGGGCAG-3' (*EcoR*I site in italics) [SEQ ID NO: 79], then the PCR product was cut with *EcoR*I. It was subjected to electrophoresis and further purified using QIAEII Gel Purification Kit (150), Qiagen. It was ligated into *P. pastoris* expression vector pPIC9 (Invitrogen) for extracellular expression. The vector pPIC9 was previously digested with *Xho*I and then treated with Klenow enzymes, and finally digested
15 with *EcoR*I. After ligation, it was transformed into *P. pastoris*. GS115 (Invitrogen), screening for recombinant BtA2 protein producing clones according to the protocols of the manufacturer. The *Pichia* host strain GS115 has a defect in the histidinol dehydrogenase gene (His4) which prevents it from synthesizing histidine. All expression plasmids carry the wild type His4 gene which complement His4 in the host, so transformants are selected
20 for their ability to grow on histidine-deficient medium. Transformants of GS1154 with Bgl II-linearized pPIC9 construct favour recombination at the AOX1 locus. Displacement of the alcohol oxidase (AOX1) structural gene occurs at a frequency of 5-35% of the His+ transformants. By patching or replica-plating on minimal dextrose (MD) versus Minimal Methanol (MM) plates, Mut+ and Mut- (methanol utilization slow) transformants can be
25 readily distinguished. Several transformants were then grown overnight in 1 ml of BMGY* media (1% w/v yeast extract, 2% w/v peptone, 100 mM potassium phosphate, pH 6.0, 1.34% w/v yeast nitrogen base with ammonium sulfate, 0.00004% w/v biotin, 1% v/v glycerol). Overnight cultures were then transferred to a larger volume and grew to O.D. 2-6 at 600 nm wavelength to log phase. Then the cells were spun down and resuspended in
30 BMMY medium (0.5% v/v methanol replaces the glycerol of BMGY medium) with its volume reduced to 1/10 of its earlier volume in BMGY. The culture was allowed to grow

for several days. 1 ml of supernatant was taken daily from the culture to screen for recombinant protein expression and at the same time, 100% v/v methanol was added to a final concentration of 0.5% to the culture. The recombinant protein in the supernatant was analysed in a SDS-PAGE (10% w/v) (Figure 18, Gel B). A suitable clone was selected to
5 grow in large volume for mass extraction of recombinant A2 clone protein.

EXAMPLE 38

SDS-PAGE

10 Recombinant proteins were separated by normal tricine SDS-PAGE. The cathode buffer was 0.1 M Tris, 0.1 M Tricine, 0.1% w/v SDS, pH 8.25 (adjust pH with Tris or Tricine) and the anode buffer was 0.2 M Tris, pH 8.9 (adjust the pH with 6N HCL). Samples were denatured and reduced by boiling the samples in a 0.1% v/v mercaptoethanol, 4% w/v SDS, 0.01% w/v Serva glue G, 12% v/v glycerol and 50 mM Tris, pH 6.8. The samples
15 and the molecular weight marker (14-97 kD, BioRad) were applied to the gel and the electrophoresis was performed at 20 mA/gel (approximate voltage 100 Volts) until the bromophenol blue reached the bottom of the gel. Gels were stained with Coomassie brilliant blue R-250 (Sigma).

20

EXAMPLE 39

Sequence analysis

The deduced nucleotide sequence was submitted to the databases of the National Center for Biotechnology Information (NCBI), using the BLAST network server for sequence
25 homology search. The amino acids sequence homology search was carried out simultaneously.

The nucleotide sequence of the clone is 507 bp in length. This includes a linker sequence ggccagag, a 286 bp 3' untranslated region with a poly-A tail, and a 218 bp probable
30 truncated coding region for the recombination protein with a stop codon (TAA) at nucleotide residues 219-221. The deduced peptide has a cyteine rich between nucleotide

residues 70 to 200 as detected in the databases of the National Center for Biotechnology Information (NCBI), using the BLAST network server. The inferred amino acid sequence from nucleotides 9-218 indicated that this partial clone codes for a protein of 66 residues, with 8 cysteine residues in the molecule (Figure 16).

5

"Examples 40 to 47 relate to Bt5 polymorphic variants"

EXAMPLE 40

PCR amplification and cloning of Bt5 genomic gene

10

Genomic DNA sequence for Bt5 was cloned by direct PCR amplification of storage mite, *Bt* obtained from Singapore and Columbia. Briefly, numerous of mites were suspended in 1 X PCR reaction buffer (10 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-Cl (pH 8.75), 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA) and boiled at 100°C for 10 minutes.

15 The sample was cooled on ice for 10 minutes. The PCR reaction was carried out in 1 X PCR buffer containing 100 mM of dNTP, 20 pmoles of each sense and antisense primers and 2.5 units of *pfu* in a final volume of 100 µl. The sense primer and the antisense primer are 5'CCCGGATCCACAATGAAGTTCGCCATCGTTCTT3' SEQ ID NO:35 (primer) and 5'GCTCTAGATTATTGGTTTGAATATC3' SEQ ID NO:36 (primer), respectively.

20 The reaction was heated to 95°C for 5 minutes, annealed at 57°C for 2 minutes, and polymerized at 72° for 2 minutes followed by 40 cycles of amplification, each consisting of 95°C for 1 minute, 57°C for 1 minute, and 72°C for 2 minutes. The final step of the last cycle extended 72°C for 10 minutes. One hundred fold dilution of the *pfu* amplified sample was reamplified using Taq polymearse. The gel purified PCR fragment was then cloned
25 into the pCR2.1 vector using the TA cloning kit (Invitrogen). Sequence of the Bt5 genomic gene was determined by ABI PRISM™ DNA sequencing kit.

EXAMPLE 41***Preparation of antigens*****(a) *Purification of recombinant Bt5***

5

The recombinant allergens Bt5 has been purified using recombinant *E coli* as a fusion with glutathione -S-transferase (GST). The proteins were purified by affinity chromatography using glutathione agarose beads. The purified proteins were then cleaved by thrombin digestion. The cleaved proteins were separated from the GST fusion partner by another
10 round of affinity chromatography using the glutathione agarose beads and then analysed on SDS-PAGE gel. Proteins were filter-sterilized before use.

(b) *Preparation of crude mite extracts*

15 Lyophilized *Bt* mites were homogenized in the presence of liquid nitrogen. The homogenised mite proteins were extracted in PBS buffer for 48 hours at 4°C. The extracts were analyzed on SDS-PAGE gel and then filter sterilized before use.

EXAMPLE 42

20

Establishment of T cell lines from allergic individuals

Ten allergic individuals have been selected on the basis of their skin test reactivity. These individuals were skin test positive for Bt. The PBMC from these individuals have been collected and the T-cells cultures were first set up using Bt crude extracts, the second
25 round of antigen stimulation was performed with recombinant Bt5 allergens. The antigen-specific T-cell lines have been established. Two cell lines have been used for the epitope mapping using a panel of synthetic peptides.

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EXAMPLE 43***T-cell epitope mapping***

Sets of peptides covering the entire sequences of Bt5 were made by the Multipin method
5 by Chiron Mimotopes Pty., Clayton, Victoria, Australia. The purity of the peptides was
confirmed by high performance liquid chromatography. The peptides were designed to be
16-amino acid residues in length, overlapping by 10 or 13 amino acid residues. Peptides 35-
67 are Bt5 peptides designed on the basis of published sequence of Bt5. Peptides 80-96 are
the peptide variants of Bt5 (refer to Figure 20). These peptide variants contain naturally
10 occurring mutations of Bt5 proteins. Subsets of adjacent peptides were pooled for initial
screening, and only those peptide pools that were stimulatory were examined in further
detail for the identification of particular epitopes. T-cell lines specific for Bt5, were
generated from PBMC of ten allergic individuals. These cell lines were tested against the
synthetic peptides by T-cell proliferation that was measured by thymidine uptake assay.

EXAMPLE 44***Cell proliferation assay***

T-cells (1×10^5) were cultured with or without irradiated antigen presenting cells (5×10^5)
20 in a final volume of 0.2 ml in complete RPMI in the presence of varying concentration of
protein antigens. Tridium thymidine ($[^3\text{H}]\text{TdR}$) were added at 48 hours, 72 hours or 96
hours for 18 hours. Cultures were harvested and thymidine incorporation was analyzed by
liquid scintillation.

EXAMPLE 45***Analysis of Bt5 genomic sequences***

The inventors have shown that a small degree of sequence polymorphism of mite allergens
exerts a significant impact on the host immune system, including both the humoral and
30 cellular immune responses. As Bt5 is the main indoor allergen causing asthma in tropical
and subtropical countries, it will be important to examine whether there is any residue

polymorphism exists in Bt5 gene(s). In accordance with the present invention, the inventors have cloned the genomic sequences of Bt5 gene(s). By aligning the coding and intron sequences, a total number of 10 Bt5 gene variants have been identified (Figure 21). Sequences 1 to 5 and 6 to 10 representing Bt5 gene sequences obtained from Colombian and Singapore Bt mites, respectively. Like Der p 2, there is only one intron present in Bt5 (32). However, the intron of Bt5 is shorter and its location is further downstream of the first codon, ATG, than Der p 2 gene (32). The size of the intron is ranging between 54 to 56 bp with stretches of nucleotides composing of either A or T base. Among the 11 Bt5 variants, nucleotide polymorphism are mainly found within the first half of the coding sequence (Table 3). Some of the nucleotide changes resulted in some amino acid residue changes (Table 2). The residue changes can be found as the conversion of a negatively charged residue to a neutral residue (residue 2), a positive charge amino acid to a negative charge residue (residue 4), a neutral residue to a negative charge residue (residue 12), a neutral residue to a neutral residue (residues 17, 23 and 33), or a neutral residue to a positive residue (residues 34 and 11). Other types of property alteration including hydrophobic residue to hydrophobic residue (residue 34), acid residue to aliphatic residue (residue 2), basic residue to acidic residue (residue 12), aliphatic residue to aromatic residue (residue 17), amide residue to aliphatic residue (residue 23), aliphatic residue to basic residue (residues 34 and 117).

EXAMPLE 46

Sequence analysis of Bt5 cDNA clones

The first cDNA sequence for Bt5 was published by Arruda *et al.* (7), subsequently a partial sequence of Bt5 was reported by Carabolla *et al.* (16). The inventors independently isolated Bt5 cDNA clones from a cDNA library constructed using total RNA extracted from local *Bt* mites. As shown in Figures 12 and 22, the instant Bt5 cDNA sequences differ from the published sequences in two important aspects. First, the cDNA clones revealed a number of polymorphic residues that were not previously reported. Secondly, the cDNA clones have extended 5'- and 3'- untranslated regions and there was a high

degree of sequence diversity shown by the various cDNA variants in these untranslated regions (Figure 13).

EXAMPLE 47

T-cell epitope mapping

In order to evaluate the immunological impact of the sequence polymorphisms found in the various Bt5 variants, T-cell epitope mapping was performed using a panel of Bt5 synthetic peptides (Figure 20). As shown in Figure 23, the T-cell epitopes recognized by allergic subjects are largely located in regions where polymorphic residues are found. A substitution of one or two amino acid residues resulted in significant difference in T-cell response. The results suggest that these polymorphic residues are potentially important for the design and development of effective immunotherapeutic reagents for the treatment of mite allergy. It has been reported that there was a small but significant degree of sequence polymorphisms exists in Der p 1 and Der p 2, the two major allergens for *Dermatophagoides pteronyssinus* mites (32,33). Further, the polymorphic residues of Der p 2 were located in regions containing major T-epitopes(32,33). Taking together, it is clear that the sequence polymorphisms exist in these major mite allergens have significant impact on the immune responses. Therefore, it is conceivable that these polymorphic residues are potentially important for the design and development of effective immunotherapeutic reagents for the treatment of mite allergy.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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CLAIMS

1. An isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding a protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is selected from Bt11, Bt10 and BtA2, each as herein defined, or polymorphic variants thereof and a polymorphic variant of Bt5 as herein defined.
2. An isolated nucleic acid molecule according to Claim 1 wherein the mite is *Blomia tropicalis*.
3. A nucleic acid molecule according to Claim 1 or 2 comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding Bt11 having an amino acid sequence as set forth in any one of SEQ ID NO: 2 to SEQ ID NO:14 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO: 2 to SEQ ID NO:14.
4. A nucleic acid molecule according to Claim 3 comprising a nucleotide sequence encoding Bt11 wherein said nucleotide sequence is as substantially set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13, or a derivative, homologue or mimetic thereof or capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a complementary form thereof under low stringency conditions.
5. A nucleic acid molecule according to Claim 3 comprising a nucleotide sequence encoding a polymorphic variant of Bt11.
6. A nucleic acid molecule according to Claim 5 comprising a nucleotide sequence substantially as set forth in SEQ ID NO:27.
7. A nucleic acid molecule according to Claim 1 or 2 comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence

encoding Bt10 having an amino acid sequence as set forth in SEQ ID NO:29 or SEQ ID NO:31 or a derivative, homologue or mimetic thereof or SEQ ID NO:29 or SEQ ID NO:31 having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:29 or SEQ ID NO:31.

8. A nucleic acid molecule according to Claim 7 comprising a nucleotide sequence encoding Bt10 wherein said nucleotide sequence is as substantially as set forth in SEQ ID NO:28 or SEQ ID NO:30 or a nucleotide sequence having at least 45% similarity to SEQ ID NO:28 or SEQ ID NO:30 or a nucleotide sequence capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or a complementary form thereof under low stringency conditions.

9. A nucleic acid molecule according to Claim 8 comprising a nucleotide sequence encoding a polymorphic variant of Bt10.

10. A nucleic acid molecule according to Claim 1 or 2 comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding a polymorphic variant of Bt5 having an amino acid sequence as set forth in SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71.

11. A nucleic acid molecule according to Claim 10 comprising a nucleotide sequence encoding a polymorphic variant of Bt5 wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a derivative, homologue or mimetic thereof or a nucleotide sequence having at least about 45% similarity to SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a nucleotide sequence capable of hybridizing to SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a complementary form thereof under low stringency conditions.

12. A nucleic acid molecule according to Claim 1 or 2 comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding BtA2 having an amino acid sequence as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to an amino acid sequence set forth in SEQ ID NO:75.
13. A nucleic acid molecule according to Claim 12 comprising a nucleotide sequence encoding BtA2 wherein said nucleotide sequence is substantially as set forth in SEQ ID NO:74 or a derivative, homologue or mimetic thereof or a nucleotide sequence having at least about 45% similarity to SEQ ID NO:74 or a nucleotide sequence capable of hybridizing to SEQ ID NO:74.
14. A nucleic acid molecule according to Claim 12 comprising a nucleotide sequence encoding a polymorphic variant of BtA2.
15. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:1.
16. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:2.
17. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:13.
18. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:27.
19. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:28.

20. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:30.
21. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:55.
22. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:56.
23. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:57.
24. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:58.
25. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:59.
26. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:60.
27. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:61.
28. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:62.
29. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:63.

30. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:64.
31. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:65.
32. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:66.
33. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:67.
34. An isolated cDNA molecule comprising a sequence of nucleotides as set forth in SEQ ID NO:74.
35. An isolated protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen wherein said protein allergen is selected from Bt11, Bt10 or BtA2, each as herein defined, or polymorphic variants thereof and a polymorphic variant of Bt5 as herein defined.
36. An isolated protein according to Claim 35 wherein the mite is *Blomia tropicalis*.
37. An isolated protein according to Claim 35 or 36 comprising an amino acid sequence defining Bt11, said amino acid sequence substantially as set forth in SEQ ID NO:3 to SEQ ID NO:12 or SEQ ID NO:14 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:3 to SEQ ID NO:12 or SEQ ID NO:14.
38. An isolated protein according to Claim 37 comprising an amino acid sequence defining Bt11, said amino acid sequence encoded by the nucleotide sequence set

forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:13 or a complementary form thereof under low stringency conditions.

39. An isolated protein according to Claim 35 or 36 comprising an amino acid sequence defining Bt10, said amino acid sequence substantially as set forth in SEQ ID NO:28 or SEQ ID NO:31 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:28 or SEQ ID NO:31.

40. An isolated protein according to Claim 39 comprising an amino acid sequence defining Bt10, said amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:28 or SEQ ID NO:30 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or a complementary form thereof under low stringency conditions.

41. An isolated protein according to Claim 35 or 36 comprising an amino acid sequence defining a polymorphic variant of Bt5, said amino acid sequence substantially as set forth in SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:49 to SEQ ID NO:53 or SEQ ID NO:68 to SEQ ID NO:71.

42. An isolated protein according to Claim 41 comprising an amino acid sequence defining a polymorphic variant of Bt5, said amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:38 to SEQ ID NO:40 or SEQ ID NO:55 to SEQ ID NO:67 or a complementary form thereof under low stringency conditions.

43. An isolated protein according to Claim 35 or 36 comprising an amino acid sequence defining BtA2, said amino acid sequence substantially as set forth in SEQ ID NO:75 or a derivative, homologue or mimetic thereof or having at least about 45% similarity to an amino acid sequence set forth in SEQ ID NO:75.
44. An isolated protein according to Claim 43 comprising an amino acid sequence defined BtA2, said amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO:74 or a derivative, homologue or mimetic thereof or a nucleotide sequence capable of hybridizing to SEQ ID NO:74 or a complementary form thereof under low stringency conditions.
45. An isolated protein comprising the amino acid sequence set forth in any one of SEQ ID NO:2, SEQ ID NO:3 to SEQ ID NO:12, SEQ ID NO:13 or SEQ ID NO:14.
46. An isolated protein comprising the amino acid sequence set forth in SEQ ID NO:29 or SEQ ID NO:31.
47. An isolated protein comprising the amino acid sequence set forth in SEQ ID NO:49.
48. An isolated protein comprising the amino acid sequence set forth in SEQ ID NO:50.
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67. An isolated protein comprising the amino acid sequence set forth in SEQ ID NO:70.
68. An isolated protein comprising the amino acid sequence set forth in SEQ ID NO:70.
69. An isolated protein comprising the amino acid sequence set forth in SEQ ID NO:75.
70. A method of preventing, reducing or otherwise ameliorating a *B. tropicalis* hypersensitivity condition in a subject, said method comprising administering to said subject an effective amount of an allergen from said *B. tropicalis* or a derivative, homologue, analogue, mimetic or chemical equivalent thereof for a time and under conditions sufficient to desensitize said individual.

71. A method of modulating, in a subject, an immune response directed to *B. tropicalis* allergen, said method comprising administering to said subject an effective amount of said allergen or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient to up-regulate, down-regulate or otherwise modulate said immune response.
72. A method for the prophylactic treatment of an allergic condition comprising the step of administering to an individual a nucleic acid molecule comprising a gene encoding Bt11 or a derivative or homologue thereof whereby airway hyper-reactivity or airway inflammation is prevented.
73. A method according to Claim 74 wherein the allergic condition is allergic asthma, atopic dermatitis and/or rhinitis.
74. A method according to Claim 72 or 73 wherein the nucleic acid molecule is in the form of an eukaryotic expression vector.
75. The method of Claim 72 wherein the eukaryotic expression vector is administered in a pharmaceutical composition comprising a carrier selected from the group consisting of normal saline and a liposome.
76. The method of Claim 72 wherein the individual is a human.
77. The method of Claim 72 wherein the vector is a plasmid vector.
78. A method according to Claim 72 wherein the administration is *via* intramuscular injection.
79. Use of a *B. tropicalis* allergen in the manufacture of a medicament for modulating an immune response.

80. An agent useful for modulating an immune response, said agent comprising a *B. tropicalis* allergen.
81. A composition for use in modulating an immune response comprising a *B. tropicalis* allergen and one or more pharmaceutically acceptable carriers and/or diluents.
82. Antibodies to a *B. tropicalis* allergen and its derivatives, homologues, analogues, mimetics and chemical equivalents thereof.
83. A method for detecting antibody directed to all or part of *B. tropicalis* allergen in a biological sample from a subject, said method comprising contacting said biological sample with *B. tropicalis* allergen or a derivative, homologue, analogue, chemical equivalent or mimetic thereof for a time and under conditions sufficient for an antibody-protein complex to form, and then detecting said complex.
84. A pharmaceutical composition useful for modulating an immune response directed to *B. tropicalis*, said composition comprising an allergen from *B. tropicalis* or derivative, homologue, analogue, chemical equivalent or mimetic thereof and one or more pharmaceutically acceptable carriers and/or diluents.

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1 5 10 15
Tyr Ile His Thr Tyr Thr Leu Thr Thr His Ile Arg Pro Val Pro Ser
20 25 30
Gly Val His Cys Ile Arg Lys Ser Leu Thr Ser Ser Ser Pro Glu Ala
35 40 45
Glu

<210> 5
<211> 13
<212> PRT
<213> Blomia tropicalis

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<400> 5

Thr Ile Asn Glu Ile Ser Leu Ile Leu Leu Leu Lys His

1

5

10

<210> 6

<211> 14

<212> PRT

<213> *Blomia tropicalis*

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Thr Pro Phe Tyr Thr Asn Leu Gln Val Tyr Thr Ile Phe Gln

1

5

10

<210> 7

<211> 4

<212> PRT

<213> *Blomia tropicalis*

<400> 7

Asp Ile Arg Glu

1

<210> 8

<211> 3

<212> PRT

<213> *Blomia tropicalis*

<400> 8

Asn Gln Tyr

1

<210> 9

<211> 5

<212> PRT

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<213> Blomia tropicalis

<400> 9

Phe Asp Leu Lys Gln

1 5

<210> 10

<211> 878

<212> PRT

<213> Blomia tropicalis

<400> 10

Ser Thr Thr Met Ala Ala Arg Ser Ala Lys Tyr Met Tyr Gln Ser Ser

1 5 10 15

Arg Ala Gly His Gly Gly Asp Ile Ser Ile Glu Tyr Gly Thr Asp Leu

20 25 30

Gly Ala Leu Thr Arg Leu Glu Asp Lys Ile Arg Leu Leu Ser Glu Asp

35 40 45

Leu Glu Ser Glu Arg Glu Leu Arg Gln Arg Val Glu Arg Glu Lys Ser

50 55 60

Asp Ile Thr Val Gln Leu Met Asn Leu Thr Glu Arg Leu Glu Glu Thr

65 70 75 80

Glu Gly Ser Ser Glu Ser Val Thr Glu Met Asn Lys Lys Arg Asp Ser

85 90 95

Glu Leu Ala Lys Leu Arg Lys Leu Leu Glu Asp Val His Met Glu Ser

100 105 110

Glu Glu Thr Ala His His Leu Arg Gln Lys His Gln Ala Ala Ile Gln

115 120 125

Glu Met Gln Asp Gln Leu Asp Gln Val Gln Lys Ala Lys Asn Lys Ser

130 135 140

Asp Lys Glu Lys Gln Lys Phe Gln Ala Glu Val Phe Glu Leu Leu Ala

145 150 155 160

Gln Val Glu Thr Ala Asn Lys Asp Lys Leu Val Ala Gln Lys Thr Val

165 170 175

Glu Lys Leu Glu Tyr Thr Val His Glu Leu Asn Ile Lys Ile Glu Glu

180 185 190

Ile Asn Arg Thr Val Val Glu Val Thr Ala His Arg Gln Arg Leu Ser

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195	200	205
Gln Glu Asn Ser Glu Leu Ile Lys Glu Val His Glu Tyr Lys Ile Ser		
210	215	220
Leu Asp Asn Ala Asn His Leu Lys Gly Gln Ile Ala Gln Gln Leu Glu		
225	230	235
Asp Thr Arg His Arg Leu Glu Asp Glu Glu Arg Lys Arg Ser Ser Leu		
	245	250
		255
Glu Asn His Ala His Thr Leu Glu Val Glu Leu Glu Ser Leu Lys Val		
	260	265
		270
Gln Leu Glu Glu Glu Ser Glu Ala Arg Leu Glu Leu Glu Arg Gln Leu		
	275	280
		285
Thr Lys Ala Asn Gly Asp Ala Ala Ser Trp Lys Ser Lys Tyr Glu Ala		
	290	295
		300
Glu Leu Gln Ala His Val Asp Glu Val Glu Glu Leu Arg Arg Lys Met		
	305	310
		315
Ala Gln Lys Ile Ser Glu Tyr Gly Glu Gln Leu Glu Ala Leu Leu Asn		
	325	330
		335
Lys Cys Ser Ala Leu Glu Lys Gln Lys Ala Arg Leu Gln Ser Glu Val		
	340	345
		350
Glu Val Leu Ile Met Asp Leu Glu Lys Ala Thr Ala His Ala Gln Ala		
	355	360
		365
Leu Glu Lys Arg Val Ser Gln Leu Glu Lys Ile Asn Leu Asp Leu Lys		
	370	375
		380
Ser Lys Leu Glu Glu Val Ser Met Leu Leu Glu Gln Thr Gln Lys Asp		
	385	390
		395
Leu Arg Val Lys Ile Ala Asp Leu Gln Lys Leu Gln His Glu Tyr Glu		
	405	410
		415
Lys Leu Arg Asp Gln Lys Glu Ala Leu Ala Arg Glu Asn Lys Lys Leu		
	420	425
		430
Ala Asp Asp Leu Ala Glu Ala Lys Ser Gln Leu Asn Asp Ala His Arg		
	435	440
		445
Arg Ile His Glu Gln Glu Ile Glu Ile Lys Arg Leu Glu Asn Glu Arg		
	450	455
		460
Glu Glu Leu Ala Ala Ala Tyr Lys Glu Ala Glu Thr Leu Arg Lys Gln		
	465	470
		475
Glu Glu Ala Lys Asn Gln Arg Leu Thr Ala Glu Leu Ala Gln Thr Arg		
	485	490
		495
His Asp Tyr Glu Lys Arg Leu Ala Gln Lys Glu Glu Glu Ile Glu Ala		

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500	505	510
Leu Arg Lys Gln Tyr Gln Ile Glu Ile Glu Gln Leu Asn Met Arg Leu		
515	520	525
Ala Glu Ala Glu Ala Lys Leu Lys Thr Glu Val Ala Arg Leu Lys Lys		
530	535	540
Lys Tyr Gln Ala Gln Ile Thr Glu Leu Glu Leu Ser Leu Asp Ala Ala		
545	550	555
Asn Lys Ala Asn Ile Asp Leu Gln Lys Thr Ile Lys Lys Gln Ala Leu		
565	570	575
Gln Ile Thr Gly Leu Gln Ala His Tyr Asp Glu Val His Arg Gln Leu		
580	585	590
Gln Gln Ala Val Asp Gln Leu Gly Val Thr Gln Arg Arg Cys Gln Ala		
595	600	605
Leu Thr Ala Glu Leu Glu Glu Met Arg Val Asn Leu Glu Gln Ala Leu		
610	615	620
Arg Ala Lys Arg Ala Ala Glu Gln Met His Glu Glu Ala Val Val Arg		
625	630	635
Val Asn Glu Leu Thr Thr Ile Asn Val Asn Leu Ala Ser Ala Lys Ser		
645	650	655
Lys Leu Glu Thr Glu Phe Ser Ala Leu Gln Asn Asp Tyr Asp Glu Val		
660	665	670
His Lys Glu Leu Arg Ile Ser Asp Glu Arg Val Gln Lys Leu Thr Ile		
675	680	685
Glu Val Lys Ser Thr Lys Asp Leu Leu Glu Ser Glu Thr Glu Arg Val		
690	695	700
Thr Lys Leu Glu Thr Ile Lys Lys Ser Leu Glu Thr Glu Val Arg Asn		
705	710	715
Leu Gln Ile Arg Ile Glu Glu Val Glu Ala Asn Ala Leu Ala Gly Gly		
725	730	735
Lys Arg Val Ile Ala Lys Leu Glu Ser Arg Ile Arg Asp Val Glu Ile		
740	745	750
Glu Val Glu Glu Glu Arg Arg Arg His Ala Glu Thr Glu Lys Met Leu		
755	760	765
Arg Lys Lys Asp His Arg Val Lys Glu Leu Leu Leu Gln Asn Glu Glu		
770	775	780
Asp His Lys Gln Ile Gln Leu Leu Gln Glu Met Ser Asp Lys Leu Asn		
785	790	795
Glu Lys Val Lys Val Tyr Lys Arg Gln Met Gln Glu Gln Glu Gly Met		

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	805		810		815										
Ser	Gln	Gln	Asn	Leu	Thr	Arg	Val	Arg	Arg	Phe	Gln	Arg	Glu	Leu	Glu
	820		825		830										
Ala	Ala	Glu	Asp	Arg	Ala	Asp	Gln	Ala	Glu	Ser	Asn	Leu	Ser	Phe	Ile
	835		840		845										
Arg	Ala	Lys	His	Arg	Ser	Trp	Val	Thr	Thr	Ser	Gln	Val	Pro	Gly	Gly
	850		855		860										
Thr	Arg	Gln	Val	Phe	Val	Thr	Glu	Gln	Glu	Ser	Ser	Asn	Phe		
865			870						875						

<210> 11

<211> 32

<212> PRT

<213> *Blomia tropicalis*

<400> 11

Ile	Thr	Ala	Cys	Asn	Lys	Phe	Ile	Ile	Ser	Tyr	Lys	Ser	Asn	Asn	Asn
1				5					10				15		
Leu	Lys	Phe	Lys	Pro	Asn	Ile	Asp	Ile	Asn	Leu	Ile	Lys	Lys	Lys	Phe
			20					25					30		

<210> 12

<211> 19

<212> PRT

<213> *Blomia tropicalis*

<400> 12

Lys	Asn	His	Asn	Leu	Leu	Asn	Thr	Pro	Arg	Leu	Ala	Phe	Thr	Thr	Thr
1				5					10				15		
Ile	Thr	Thr													

<210> 13

<211> 2625

<212> DNA

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<213> Blomia tropicalis

<220>

<221> CDS

<222> (1) .. (2625)

<400> 13

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Met Ala Ala Arg Ser Ala Lys Tyr Met Tyr Gln Ser Ser Arg Ala Gly	
1 5 10 15	
 cac ggc ggc gac att tcc atc gaa tat gga acc gat ttg ggc gca ctc	96
His Gly Gly Asp Ile Ser Ile Glu Tyr Gly Thr Asp Leu Gly Ala Leu	
20 25 30	
 acc cga ctt gag gac aag att cga ctt ttg tcc gaa gat ttg gaa agc	144
Thr Arg Leu Glu Asp Lys Ile Arg Leu Leu Ser Glu Asp Leu Glu Ser	
35 40 45	
 gaa cgg gag ctt cga caa cgt gtt gaa cga gaa aag tcg gat att acc	192
Glu Arg Glu Leu Arg Gln Arg Val Glu Arg Glu Lys Ser Asp Ile Thr	
50 55 60	
 gtt caa ttg atg aac ttg acc gaa cgt ttg gag gag acc gaa ggt tcg	240
Val Gln Leu Met Asn Leu Thr Glu Arg Leu Glu Glu Thr Glu Gly Ser	
65 70 75 80	
 agc gaa agc gtt acc gag atg aac aag aaa cga gac tcg gaa ttg gct	288
Ser Glu Ser Val Thr Glu Met Asn Lys Lys Arg Asp Ser Glu Leu Ala	
85 90 95	
 aag ctt cga aag ttg ctc gaa gat gtc cat atg gaa agc gag gag act	336
Lys Leu Arg Lys Leu Leu Glu Asp Val His Met Glu Ser Glu Glu Thr	
100 105 110	
 gct cac cat ttg aga caa aag cac caa gct gcc att caa gag atg caa	384
Ala His His Leu Arg Gln Lys His Gln Ala Ala Ile Gln Glu Met Gln	
115 120 125	

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gat caa ttg gat cag gtg caa aag gca aag aac aag tcg gat aag gag	432
Asp Gln Leu Asp Gln Val Gln Lys Ala Lys Asn Lys Ser Asp Lys Glu	
130 135 140	
aag caa aag ttc caa gcc gaa gtt ttc gaa ttg ttg gct cag gtt gag	480
Lys Gln Lys Phe Gln Ala Glu Val Phe Glu Leu Leu Ala Gln Val Glu	
145 150 155 160	
act gcc aac aag gac aag ttg gtc gct cag aag acg gta gaa aag ttg	528
Thr Ala Asn Lys Asp Lys Leu Val Ala Gln Lys Thr Val Glu Lys Leu	
165 170 175	
gaa tac acc gta cat gag ttg aac att aag att gaa gaa atc aat cga	576
Glu Tyr Thr Val His Glu Leu Asn Ile Lys Ile Glu Glu Ile Asn Arg	
180 185 190	
act gtt gtt gag gtt acc gct cac cga caa cga ttg tcg caa gag aat	624
Thr Val Val Glu Val Thr Ala His Arg Gln Arg Leu Ser Gln Glu Asn	
195 200 205	
tcg gaa ttg atc aag gaa gtt cat gaa tac aag att tca ttg gac aat	672
Ser Glu Leu Ile Lys Glu Val His Glu Tyr Lys Ile Ser Leu Asp Asn	
210 215 220	
gct aac cac ttg aag gga caa att gca caa caa ttg gag gac acc cga	720
Ala Asn His Leu Lys Gly Gln Ile Ala Gln Gln Leu Glu Asp Thr Arg	
225 230 235 240	
cac cga ttg gag gat gaa gaa cga aaa cga tca agc ttg gag aac cac	768
His Arg Leu Glu Asp Glu Glu Arg Lys Arg Ser Ser Leu Glu Asn His	
245 250 255	
gcc cac aca ttg gaa gtt gaa ttg gaa tca ttg aag gta caa ttg gaa	816
Ala His Thr Leu Glu Val Glu Leu Glu Ser Leu Lys Val Gln Leu Glu	
260 265 270	
gag gaa tct gag gcc cga tta gaa ttg gaa cga caa ttg act aaa gct	864

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Glu Glu Ser Glu Ala Arg Leu Glu Leu Glu Arg Gln Leu Thr Lys Ala	
275	280 285
aat ggc gat gct gct tca tgg aaa tcc aaa tac gaa gcc gaa ctt caa	912
Asn Gly Asp Ala Ala Ser Trp Lys Ser Lys Tyr Glu Ala Glu Leu Gln	
290	295 300
gct cac gtt gat gag gtt gaa gaa ctt cga cga aag atg gcc cag aag	960
Ala His Val Asp Glu Val Glu Glu Leu Arg Arg Lys Met Ala Gln Lys	
305	310 315 320
att tcg gaa tat ggg gaa caa ttg gaa gca ttg ttg aac aaa tgt agt	1008
Ile Ser Glu Tyr Gly Glu Gln Leu Glu Ala Leu Leu Asn Lys Cys Ser	
	325 330 335
gca ttg gaa aag caa aag gct cga ctc caa tcg gag gta gaa gtt ttg	1056
Ala Leu Glu Lys Gln Lys Ala Arg Leu Gln Ser Glu Val Glu Val Leu	
	340 345 350
atc atg gac ttg gaa aag gct acc gca cat gcc cag gca ttg gag aaa	1104
Ile Met Asp Leu Glu Lys Ala Thr Ala His Ala Gln Ala Leu Glu Lys	
	355 360 365
cgc gtt tcc caa ttg gag aag atc aac ctc gac ctc aag agc aag ttg	1152
Arg Val Ser Gln Leu Glu Lys Ile Asn Leu Asp Leu Lys Ser Lys Leu	
	370 375 380
gag gag gtc agc atg ttg ttg gaa caa act caa aag gat ctc cgt gtc	1200
Glu Glu Val Ser Met Leu Leu Glu Gln Thr Gln Lys Asp Leu Arg Val	
	385 390 395 400
aag att gca gac ttg cag aaa ttg caa cac gag tac gaa aag ttg cga	1248
Lys Ile Ala Asp Leu Gln Lys Leu Gln His Glu Tyr Glu Lys Leu Arg	
	405 410 415
gac caa aag gaa gca ttg gcc cgt gag aac aag aaa ctc gct gat gat	1296
Asp Gln Lys Glu Ala Leu Ala Arg Glu Asn Lys Lys Leu Ala Asp Asp	
	420 425 430

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ttg gct gaa gct aaa tct cag ttg aat gac gcc cac cga cga atc cat 1344
 Leu Ala Glu Ala Lys Ser Gln Leu Asn Asp Ala His Arg Arg Ile His
 435 440 445

gaa caa gag atc gag atc aaa cga ttg gag aac gag cga gaa gaa ttg 1392
 Glu Gln Glu Ile Glu Ile Lys Arg Leu Glu Asn Glu Arg Glu Glu Leu
 450 455 460

gct gcc gca tac aag gaa gct gaa acc ttg cga aaa caa gaa gag gca 1440
 Ala Ala Ala Tyr Lys Glu Ala Glu Thr Leu Arg Lys Gln Glu Glu Ala
 465 470 475 480

aag aat caa cga ttg act gcc gag ttg gca cag aca cga cac gac tat 1488
 Lys Asn Gln Arg Leu Thr Ala Glu Leu Ala Gln Thr Arg His Asp Tyr
 485 490 495

gag aaa cga ttg gca caa aag gaa gag gag att gaa gct ctt cga aaa 1536
 Glu Lys Arg Leu Ala Gln Lys Glu Glu Glu Ile Glu Ala Leu Arg Lys
 500 505 510

caa tac caa att gaa att gaa caa ttg aac atg cga ttg gct gag gct 1584
 Gln Tyr Gln Ile Glu Ile Glu Gln Leu Asn Met Arg Leu Ala Glu Ala
 515 520 525

gaa gct aaa ttg aag acc gag gtt gca cga ctc aag aag aag tac caa 1632
 Glu Ala Lys Leu Lys Thr Glu Val Ala Arg Leu Lys Lys Lys Tyr Gln
 530 535 540

gca caa atc act gaa ttg gaa ttg tcg ttg gat gcc gct aat aag gct 1680
 Ala Gln Ile Thr Glu Leu Glu Leu Ser Leu Asp Ala Ala Asn Lys Ala
 545 550 555 560

aac atc gac ttg cag aag acg atc aag aag caa gct ctc caa atc act 1728
 Asn Ile Asp Leu Gln Lys Thr Ile Lys Lys Gln Ala Leu Gln Ile Thr
 565 570 575

ggg ctt caa gcc cat tat gat gag gta cat cgt caa ttg caa caa gct 1776

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Gly Leu Gln Ala His Tyr Asp Glu Val His Arg Gln Leu Gln Gln Ala	
580	585
590	
ggt gat caa ttg ggt gtc act caa cga cga tgc caa gca ttg acc gcc	1824
Val Asp Gln Leu Gly Val Thr Gln Arg Arg Cys Gln Ala Leu Thr Ala	
595	600
605	
gaa ttg gag gag atg cga gtc aac ttg gaa caa gca ctt cgt gct aaa	1872
Glu Leu Glu Glu Met Arg Val Asn Leu Glu Gln Ala Leu Arg Ala Lys	
610	615
620	
cga gct gcc gaa caa atg cac gaa gag gca gtt gtc cga gtc aac gaa	1920
Arg Ala Ala Glu Gln Met His Glu Glu Ala Val Val Arg Val Asn Glu	
625	630
635	640
ctt aca acc atc aat gtc aac ctc gct tcg gcc aag agc aaa ttg gag	1968
Leu Thr Thr Ile Asn Val Asn Leu Ala Ser Ala Lys Ser Lys Leu Glu	
645	650
655	
act gaa ttc tct gct ctt caa aac gat tac gat gaa gta cac aag gaa	2016
Thr Glu Phe Ser Ala Leu Gln Asn Asp Tyr Asp Glu Val His Lys Glu	
660	665
670	
ctc cga atc tct gac gaa cga gtg caa aaa ctt acc att gag gtc aag	2064
Leu Arg Ile Ser Asp Glu Arg Val Gln Lys Leu Thr Ile Glu Val Lys	
675	680
685	
tcg aca aag gac ttg ctc gaa tca gaa acg gaa cgt gtc aca aaa ttg	2112
Ser Thr Lys Asp Leu Leu Glu Ser Glu Thr Glu Arg Val Thr Lys Leu	
690	695
700	
gaa aca atc aag aaa tca ttg gaa acc gaa gta cga aat ctc caa atc	2160
Glu Thr Ile Lys Lys Ser Leu Glu Thr Glu Val Arg Asn Leu Gln Ile	
705	710
715	720
cga att gaa gaa gtt gag gct aat gca ttg gct ggt ggt aaa cga gtc	2208
Arg Ile Glu Glu Val Glu Ala Asn Ala Leu Ala Gly Gly Lys Arg Val	
725	730
735	

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atc gcc aag ttg gaa tcg cga atc cgt gat gtt gag att gag gtt gaa	2256
Ile Ala Lys Leu Glu Ser Arg Ile Arg Asp Val Glu Ile Glu Val Glu	
740 745 750	
 gaa gaa cga cga cga cac gca gaa act gaa aag atg ctt cga aag aag	2304
Glu Glu Arg Arg Arg His Ala Glu Thr Glu Lys Met Leu Arg Lys Lys	
755 760 765	
 gat cat cgt gtc aag gaa ttg ttg ctt caa aac gag gag gac cat aaa	2352
Asp His Arg Val Lys Glu Leu Leu Leu Gln Asn Glu Glu Asp His Lys	
770 775 780	
 caa att caa ttg ttg caa gaa atg tcc gat aaa ttg aac gaa aag gtc	2400
Gln Ile Gln Leu Leu Gln Glu Met Ser Asp Lys Leu Asn Glu Lys Val	
785 790 795 800	
 aag gtt tac aaa cga caa atg caa gaa cag gag gga atg agc caa caa	2448
Lys Val Tyr Lys Arg Gln Met Gln Glu Gln Glu Gly Met Ser Gln Gln	
805 810 815	
 aac ttg acc cgt gtc cga cga ttc caa cga gag ttg gaa gcc gcc gaa	2496
Asn Leu Thr Arg Val Arg Arg Phe Gln Arg Glu Leu Glu Ala Ala Glu	
820 825 830	
 gac cga gct gat caa gct gaa tcc aat ttg tcg ttc atc cga gcc aaa	2544
Asp Arg Ala Asp Gln Ala Glu Ser Asn Leu Ser Phe Ile Arg Ala Lys	
835 840 845	
 cat cga tcg tgg gtc acc acc agt caa gtt cct ggc ggt act cga caa	2592
His Arg Ser Trp Val Thr Thr Ser Gln Val Pro Gly Gly Thr Arg Gln	
850 855 860	
 gtc ttt gtc acg gaa caa gag agc tcg aac ttt	2625
Val Phe Val Thr Glu Gln Glu Ser Ser Asn Phe	
865 870 875	

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<210> 14

<211> 875

<212> PRT

<213> Blomia tropicalis

<400> 14

Met Ala Ala Arg Ser Ala Lys Tyr Met Tyr Gln Ser Ser Arg Ala Gly
1 5 10 15

His Gly Gly Asp Ile Ser Ile Glu Tyr Gly Thr Asp Leu Gly Ala Leu
20 25 30

Thr Arg Leu Glu Asp Lys Ile Arg Leu Leu Ser Glu Asp Leu Glu Ser
35 40 45

Glu Arg Glu Leu Arg Gln Arg Val Glu Arg Glu Lys Ser Asp Ile Thr
50 55 60

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

Ser Glu Ser Val Thr Glu Met Asn Lys Lys Arg Asp Ser Glu Leu Ala
85 90 95

Lys Leu Arg Lys Leu Leu Glu Asp Val His Met Glu Ser Glu Glu Thr
100 105 110

Ala His His Leu Arg Gln Lys His Gln Ala Ala Ile Gln Glu Met Gln
115 120 125

Asp Gln Leu Asp Gln Val Gln Lys Ala Lys Asn Lys Ser Asp Lys Glu
130 135 140

Lys Gln Lys Phe Gln Ala Glu Val Phe Glu Leu Leu Ala Gln Val Glu
145 150 155 160

Thr Ala Asn Lys Asp Lys Leu Val Ala Gln Lys Thr Val Glu Lys Leu
165 170 175

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Glu Tyr Thr Val His Glu Leu Asn Ile Lys Ile Glu Glu Ile Asn Arg
180 185 190

Thr Val Val Glu Val Thr Ala His Arg Gln Arg Leu Ser Gln Glu Asn
195 200 205

Ser Glu Leu Ile Lys Glu Val His Glu Tyr Lys Ile Ser Leu Asp Asn
210 215 220

Ala Asn His Leu Lys Gly Gln Ile Ala Gln Gln Leu Glu Asp Thr Arg
225 230 235 240

His Arg Leu Glu Asp Glu Glu Arg Lys Arg Ser Ser Leu Glu Asn His
245 250 255

Ala His Thr Leu Glu Val Glu Leu Glu Ser Leu Lys Val Gln Leu Glu
260 265 270

Glu Glu Ser Glu Ala Arg Leu Glu Leu Glu Arg Gln Leu Thr Lys Ala
275 280 285

Asn Gly Asp Ala Ala Ser Trp Lys Ser Lys Tyr Glu Ala Glu Leu Gln
290 295 300

Ala His Val Asp Glu Val Glu Glu Leu Arg Arg Lys Met Ala Gln Lys
305 310 315 320

Ile Ser Glu Tyr Gly Glu Gln Leu Glu Ala Leu Leu Asn Lys Cys Ser
325 330 335

Ala Leu Glu Lys Gln Lys Ala Arg Leu Gln Ser Glu Val Glu Val Leu
340 345 350

Ile Met Asp Leu Glu Lys Ala Thr Ala His Ala Gln Ala Leu Glu Lys
355 360 365

Arg Val Ser Gln Leu Glu Lys Ile Asn Leu Asp Leu Lys Ser Lys Leu

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370	375	380
Glu Glu Val Ser Met Leu Leu Glu Gln Thr Gln Lys Asp Leu Arg Val		
385	390	395 400
Lys Ile Ala Asp Leu Gln Lys Leu Gln His Glu Tyr Glu Lys Leu Arg		
	405	410 415
Asp Gln Lys Glu Ala Leu Ala Arg Glu Asn Lys Lys Leu Ala Asp Asp		
	420	425 430
Leu Ala Glu Ala Lys Ser Gln Leu Asn Asp Ala His Arg Arg Ile His		
	435	440 445
Glu Gln Glu Ile Glu Ile Lys Arg Leu Glu Asn Glu Arg Glu Glu Leu		
	450	455 460
Ala Ala Ala Tyr Lys Glu Ala Glu Thr Leu Arg Lys Gln Glu Glu Ala		
465	470	475 480
Lys Asn Gln Arg Leu Thr Ala Glu Leu Ala Gln Thr Arg His Asp Tyr		
	485	490 495
Glu Lys Arg Leu Ala Gln Lys Glu Glu Glu Ile Glu Ala Leu Arg Lys		
	500	505 510
Gln Tyr Gln Ile Glu Ile Glu Gln Leu Asn Met Arg Leu Ala Glu Ala		
	515	520 525
Glu Ala Lys Leu Lys Thr Glu Val Ala Arg Leu Lys Lys Lys Tyr Gln		
	530	535 540
Ala Gln Ile Thr Glu Leu Glu Leu Ser Leu Asp Ala Ala Asn Lys Ala		
545	550	555 560
Asn Ile Asp Leu Gln Lys Thr Ile Lys Lys Gln Ala Leu Gln Ile Thr		
	565	570 575

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Gly Leu Gln Ala His Tyr Asp Glu Val His Arg Gln Leu Gln Gln Ala

580

585

590

Val Asp Gln Leu Gly Val Thr Gln Arg Arg Cys Gln Ala Leu Thr Ala

595

600

605

Glu Leu Glu Glu Met Arg Val Asn Leu Glu Gln Ala Leu Arg Ala Lys

610

615

620

Arg Ala Ala Glu Gln Met His Glu Glu Ala Val Val Arg Val Asn Glu

625

630

635

640

Leu Thr Thr Ile Asn Val Asn Leu Ala Ser Ala Lys Ser Lys Leu Glu

645

650

655

Thr Glu Phe Ser Ala Leu Gln Asn Asp Tyr Asp Glu Val His Lys Glu

660

665

670

Leu Arg Ile Ser Asp Glu Arg Val Gln Lys Leu Thr Ile Glu Val Lys

675

680

685

Ser Thr Lys Asp Leu Leu Glu Ser Glu Thr Glu Arg Val Thr Lys Leu

690

695

700

Glu Thr Ile Lys Lys Ser Leu Glu Thr Glu Val Arg Asn Leu Gln Ile

705

710

715

720

Arg Ile Glu Glu Val Glu Ala Asn Ala Leu Ala Gly Gly Lys Arg Val

725

730

735

Ile Ala Lys Leu Glu Ser Arg Ile Arg Asp Val Glu Ile Glu Val Glu

740

745

750

Glu Glu Arg Arg Arg His Ala Glu Thr Glu Lys Met Leu Arg Lys Lys

755

760

765

Asp His Arg Val Lys Glu Leu Leu Leu Gln Asn Glu Glu Asp His Lys

770

775

780

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Gln Ile Gln Leu Leu Gln Glu Met Ser Asp Lys Leu Asn Glu Lys Val
785 790 795 800

Lys Val Tyr Lys Arg Gln Met Gln Glu Gln Glu Gly Met Ser Gln Gln
805 810 815

Asn Leu Thr Arg Val Arg Arg Phe Gln Arg Glu Leu Glu Ala Ala Glu
820 825 830

Asp Arg Ala Asp Gln Ala Glu Ser Asn Leu Ser Phe Ile Arg Ala Lys
835 840 845

His Arg Ser Trp Val Thr Thr Ser Gln Val Pro Gly Gly Thr Arg Gln
850 855 860

Val Phe Val Thr Glu Gln Glu Ser Ser Asn Phe
865 870 875

<210> 15

<211> 701

<212> PRT

<213> Dermatophagoides farinae

<400> 15

Met Asn Lys Lys Arg Asp Ser Glu Leu Arg Lys Leu Arg Lys Leu Leu
1 5 10 15

Glu Asp Val His Ile Glu Ser Glu Glu Thr Ala His His Leu Arg Gln
20 25 30

Lys His Gln Ala Ala Ile Gln Glu Met Gln Asp Gln Leu Asp Gln Leu
35 40 45

Gln Lys Ala Lys Asn Lys Ser Asp Lys Glu Lys Gln Lys Phe Gln Ala
50 55 60

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Glu Val Phe Glu Leu Leu Ala Gln Leu Glu Thr Ala Asn Lys Glu Lys
65 70 75 80

Leu Thr Ala Leu Lys Asn Val Glu Lys Leu Glu Tyr Thr Val His Glu
85 90 95

Leu Asn Ile Lys Ile Glu Glu Ile Asn Arg Thr Val Ile Glu Leu Thr
100 105 110

Ser His Lys Gln Arg Leu Ser Gln Glu Asn Thr Glu Leu Ile Lys Glu
115 120 125

Val His Glu Val Lys Leu Gln Leu Asp Asn Ala Asn His Leu Lys Thr
130 135 140

Gln Ile Ala Gln Gln Leu Glu Asp Thr Arg His Arg Leu Glu Glu Glu
145 150 155 160

Glu Arg Lys Arg Ala Ser Leu Glu Asn His Ala His Thr Leu Glu Val
165 170 175

Glu Leu Glu Ser Leu Lys Val Gln Leu Asp Glu Glu Ser Glu Ala Arg
180 185 190

Leu Glu Leu Glu Arg Gln Leu Thr Lys Ala Asn Gly Asp Ala Ala Ser
195 200 205

Trp Lys Ser Lys Tyr Glu Ala Glu Leu Gln Ala His Glu Asp Glu Val
210 215 220

Glu Glu Leu Arg Arg Lys Met Ala Gln Lys Ile Ser Glu Tyr Glu Glu
225 230 235 240

Gln Leu Glu Ala Leu Leu Asn Lys Cys Ser Ser Leu Glu Lys Gln Lys
245 250 255

Ser Arg Leu Gln Ser Glu Val Glu Val Leu Ile Met Asp Leu Glu Lys

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260	265	270
Ala Thr Arg His Ala Gln Gln Leu Glu Lys Arg Val Ala Gln Leu Glu		
275	280	285
Lys Ile Asn Leu Asp Leu Lys Asn Lys Leu Glu Glu Val Thr Met Leu		
290	295	300
Met Glu Gln Ala Gln Lys Glu Leu Arg Val Lys Ile Ala Glu Leu Gln		
305	310	315 320
Lys Leu Gln His Glu Tyr Glu Lys Leu Arg Asp Gln Arg His Gln Leu		
325	330	335
Ala Arg Glu Asn Lys Lys Leu Thr Asp Asp Leu Ala Glu Ala Lys Ser		
340	345	350
Gln Leu Asn Asp Ala His Arg Arg Ile His Glu Gln Glu Ile Glu Ile		
355	360	365
Lys Arg Leu Glu Asn Glu Arg Asp Glu Leu Ser Ala Ala Tyr Lys Glu		
370	375	380
Ala Glu Thr Leu Arg Lys Gln Glu Glu Ala Lys Asn Gln Arg Leu Ile		
385	390	395 400
Ala Glu Leu Ala Gln Val Arg His Asp Tyr Glu Lys Arg Leu Ala Gln		
405	410	415
Lys Asp Glu Glu Ile Glu Ala Leu Arg Lys Gln Tyr Gln Ile Glu Ile		
420	425	430
Glu Gln Leu Asn Met Arg Leu Ala Glu Ala Glu Ala Lys Leu Lys Thr		
435	440	445
Glu Ile Ala Arg Leu Lys Lys Lys Tyr Gln Ala Gln Ile Thr Glu Leu		
450	455	460

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Glu Leu Ser Leu Asp Ala Ala Asn Lys Ala Asn Ile Asp Leu Gln Lys
465 470 475 480

Thr Met Lys Lys Gln Ala Leu Gln Ile Thr Glu Leu Gln Ala His Tyr
485 490 495

Asp Glu Val His Arg Gln Leu Gln Gln Ala Val Asp Gln Leu Gly Val
500 505 510

Thr Gln Arg Arg Cys Gln Ala Leu Gln Ala Glu Leu Glu Glu Met Arg
515 520 525

Ile Ala Leu Glu Gln Ala Asn Arg Ala Lys Ala Gln Ala Glu Gln Leu
530 535 540

His Glu Glu Ala Val Val Arg Val Asn Glu Leu Thr Thr Ile Asn Val
545 550 555 560

Asn Leu Ala Ser Ala Lys Ser Lys Leu Glu Ser Glu Phe Ser Ala Leu
565 570 575

Gln Ala Asp Tyr Asp Glu Val His Lys Glu Leu Arg Ile Ser Asp Glu
580 585 590

Arg Val Gln Lys Leu Thr Ile Glu Leu Lys Ser Thr Lys Asp Leu Leu
595 600 605

Ile Glu Glu Gln Glu Arg Leu Val Lys Leu Glu Thr Val Lys Lys Ser
610 615 620

Leu Glu Gln Glu Val Arg Thr Leu His Val Arg Ile Glu Glu Val Glu
625 630 635 640

Ala Asn Ala Leu Ala Gly Gly Lys Arg Val Ile Ala Lys Leu Glu Ser
645 650 655

Arg Ile Arg Asp Val Glu Ile Glu Val Glu Glu Glu Arg Arg Arg His
660 665 670

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Ala Glu Thr Asp Lys Met Leu Ala Lys Lys Asp His Arg Val Lys Glu
675 680 685

Leu Leu Leu Gln Pro Pro Glu Arg Glu His Pro Arg Val
690 695 700

<210> 16

<211> 878

<212> PRT

<213> Drosophila melanogaster

<400> 16

Met Ser Ser Ser Gln Ala Val Arg Ser Ser Lys Tyr Ser Tyr Arg Ala
1 5 10 15

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

Asp Leu Ser Ser Leu Ser Arg Leu Glu Asp Lys Ile Arg Leu Leu Gln
35 40 45

Asp Asp Leu Glu Val Glu Arg Glu Leu Arg Gln Arg Ile Glu Arg Glu
50 55 60

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

Glu Ala Glu Gly Gly Ala Glu His Gln Phe Glu Ala Asn Arg Lys Arg
85 90 95

Asp Ala Glu Leu Leu Lys Leu Arg Lys Leu Leu Glu Asp Val His Leu
100 105 110

Glu Ser Glu Glu Thr Thr Leu Leu Leu Lys Lys Lys His Asn Glu Ile
115 120 125

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Ile Thr Asp Phe Gln Glu Gln Val Glu Ile Leu Thr Lys Asn Lys Ala

130

135

140

Arg Ala Glu Lys Asp Lys Ala Lys Phe Gln Thr Glu Val Tyr Glu Leu

145

150

155

160

Leu Ser Gln Ile Glu Ser Tyr Asn Lys Glu Lys Ile Val Ser Glu Lys

165

170

175

His Ile Ser Lys Leu Glu Val Ser Ile Ser Glu Leu Asn Val Lys Ile

180

185

190

Glu Glu Leu Asn Arg Thr Val Ile Asp Ile Ser Ser His Arg Ser Arg

195

200

205

Leu Ser Gln Glu Asn Ile Glu Leu Thr Lys Asp Val Gln Asp Leu Lys

210

215

220

Val Gln Leu Asp Thr Val Ser Phe Ser Lys Ser Gln Val Ile Ser Gln

225

230

235

240

Leu Glu Asp Ala Arg Arg Arg Leu Glu Asp Glu Asp Arg Arg Arg Ser

245

250

255

Leu Leu Glu Ser Ser Leu His Gln Val Glu Ile Glu Leu Asp Ser Val

260

265

270

Arg Asn Gln Leu Glu Glu Glu Ser Glu Ala Arg Ile Asp Leu Glu Arg

275

280

285

Gln Leu Val Lys Ala Asn Ala Asp Ala Thr Ser Trp Gln Asn Lys Trp

290

295

300

Asn Ser Glu Val Ala Ala Arg Ala Glu Glu Val Glu Glu Ile Arg Arg

305

310

315

320

Lys Tyr Gln Val Arg Ile Thr Glu Leu Glu Glu His Ile Glu Ser Leu

325

330

335

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Ile Val Lys Val Asn Asn Leu Glu Lys Met Lys Thr Arg Leu Ala Ser
340 345 350

Glu Val Glu Val Leu Ile Ile Asp Leu Glu Lys Ser Asn Asn Ser Cys
355 360 365

Arg Glu Leu Thr Lys Ser Val Asn Thr Leu Glu Lys His Asn Val Glu
370 375 380

Leu Lys Ser Arg Leu Asp Glu Thr Ile Ile Leu Tyr Glu Thr Ser Gln
385 390 395 400

Arg Asp Leu Lys Asn Lys His Ala Asp Leu Val Arg Thr Val His Glu
405 410 415

Leu Asp Lys Val Lys Asp Asn Asn Asn Gln Leu Thr Arg Glu Asn Lys
420 425 430

Lys Leu Gly Asp Asp Leu His Glu Ala Lys Gly Ala Ile Asn Glu Leu
435 440 445

Asn Arg Arg Leu His Glu Leu Glu Leu Glu Leu Arg Arg Leu Glu Asn
450 455 460

Glu Arg Asp Glu Leu Thr Ala Ala Tyr Lys Glu Ala Glu Ala Gly Arg
465 470 475 480

Lys Ala Glu Glu Gln Arg Gly Gln Arg Leu Ala Ala Asp Phe Asn Gln
485 490 495

Tyr Arg His Ala Glu Arg Arg Leu Ala Glu Lys Asp Glu Glu Ile Glu
500 505 510

Ala Ile Arg Lys Gln Thr Ser Ile Glu Ile Glu Gln Leu Asn Ala Arg
515 520 525

Val Ile Glu Ala Glu Thr Arg Leu Lys Thr Glu Val Thr Arg Ile Lys

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530	535	540	
Lys Lys Leu Gln Ile Gln Ile Thr Glu Leu Glu Met Ser Leu Asp Val			
545	550	555	560
Ala Asn Lys Thr Asn Ile Asp Leu Gln Lys Val Ile Lys Lys Gln Ser			
	565	570	575
Leu Gln Leu Thr Glu Leu Gln Ala His Tyr Glu Asp Val Gln Arg Gln			
	580	585	590
Leu Gln Ala Thr Leu Asp Gln Tyr Ala Val Ala Gln Arg Arg Leu Ala			
	595	600	605
Gly Leu Asn Gly Glu Leu Glu Glu Val Arg Ser His Leu Asp Ser Ala			
	610	615	620
Asn Arg Ala Lys Arg Thr Val Glu Leu Gln Tyr Glu Glu Ala Ala Ser			
625	630	635	640
Arg Ile Asn Glu Leu Thr Thr Ala Asn Val Ser Leu Val Ser Ile Lys			
	645	650	655
Ser Lys Leu Glu Gln Glu Leu Ser Val Val Ala Ser Asp Tyr Glu Glu			
	660	665	670
Val Ser Lys Glu Leu Arg Ile Ser Asp Glu Arg Tyr Gln Lys Val Gln			
	675	680	685
Val Glu Leu Lys His Val Val Glu Gln Val His Glu Glu Gln Glu Arg			
	690	695	700
Ile Val Lys Leu Glu Thr Ile Lys Lys Ser Leu Glu Val Glu Val Lys			
705	710	715	720
Asn Leu Ser Ile Arg Leu Glu Glu Val Glu Leu Asn Ala Val Ala Gly			
	725	730	735

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Ser Lys Arg Ile Ile Ser Lys Leu Glu Ala Arg Ile Arg Asp Leu Glu
740 745 750

Leu Glu Leu Glu Glu Glu Lys Arg Arg His Ala Glu Thr Ile Lys Ile
755 760 765

Leu Arg Lys Lys Glu Arg Thr Val Lys Glu Val Leu Val Gln Cys Glu
770 775 780

Glu Asp Gln Lys Asn Leu Ile Leu Leu Gln Asp Ala Leu Asp Lys Ser
785 790 795 800

Thr Ala Lys Ile Asn Ile Tyr Arg Arg Gln Leu Ser Glu Gln Glu Gly
805 810 815

Val Ser Gln Gln Thr Thr Thr Arg Val Arg Arg Phe Gln Arg Glu Leu
820 825 830

Glu Ala Ala Glu Asp Arg Ala Asp Thr Ala Glu Ser Ser Leu Asn Ile
835 840 845

Ile Arg Ala Lys His Arg Thr Phe Val Thr Thr Ser Thr Val Pro Gly
850 855 860

Ser Gln Val Tyr Ile Gln Glu Thr Thr Arg Thr Ile Thr Glu
865 870 875

<210> 17

<211> 891

<212> PRT

<213> Onchocerca volvulus

<400> 17

Met Ser Gly Ser Leu Tyr Arg Ser Pro Ser Ala Ala Leu Tyr Lys Ser
1 5 10 15

Pro Ser Met Ser Ala Phe Ser Gly Leu Pro Ala Ala Phe Gly Ser Met

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

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Asp Gln Lys Val Gln Leu Asp Asn Leu Gln His Val Lys Tyr Gln Leu
225 230 235 240

Ala Gln Gln Leu Glu Glu Ala Arg Arg Arg Leu Glu Asp Ala Glu Arg
245 250 255

Glu Arg Ser Gln Leu Gln Ala Gln Leu His Gln Val Gln Leu Glu Leu
260 265 270

Asp Ser Val Arg Thr Ala Leu Asp Glu Glu Ser Ala Ala Arg Ala Glu
275 280 285

Ala Glu His Lys Leu Ala Leu Ala Asn Thr Glu Ile Thr Gln Trp Lys
290 295 300

Ser Lys Phe Asp Ala Glu Val Ala Leu His His Glu Glu Val Glu Asp
305 310 315 320

Leu Arg Lys Lys Met Leu Gln Lys Gln Ala Glu Tyr Glu Glu Gln Ile
325 330 335

Glu Ile Met Leu Gln Lys Ile Ser Gln Leu Glu Lys Ala Lys Ser Arg
340 345 350

Leu Gln Ser Glu Val Glu Val Leu Ile Val Asp Leu Glu Lys Ala Gln
355 360 365

Asn Thr Ile Ala Ile Leu Glu Arg Ala Lys Glu Gln Leu Glu Lys Thr
370 375 380

Val Met Glu Leu Lys Val Arg Ile Asp Glu Leu Thr Val Glu Leu Glu
385 390 395 400

Ala Ala Gln Arg Glu Ala Arg Ala Ala Leu Ala Glu Leu Gln Lys Met
405 410 415

Lys Asn Leu Tyr Glu Lys Ala Val Glu Gln Lys Glu Ala Leu Ala Arg
420 425 430

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Glu Asn Lys Lys Leu Gln Asp Asp Leu His Glu Ala Lys Glu Ala Leu
435 440 445

Ala Asp Ala Asn Arg Lys Leu His Glu Leu Asp Leu Glu Asn Ala Arg
450 455 460

Leu Ala Gly Glu Ile Arg Glu Leu Gln Thr Ala Leu Lys Glu Ser Glu
465 470 475 480

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

Leu Gln Gln Leu Arg Ile Glu Met Glu Arg Arg Leu Gln Glu Lys Glu
500 505 510

Glu Glu Met Glu Ala Leu Arg Lys Asn Met Gln Phe Glu Ile Asp Arg
515 520 525

Leu Thr Ala Ala Leu Ala Asp Ala Glu Ala Arg Met Lys Ala Glu Ile
530 535 540

Ala Arg Leu Lys Lys Lys Tyr Gln Ala Glu Ile Ala Glu Leu Glu Met
545 550 555 560

Thr Val Asp Asn Leu Asn Arg Ala Asn Ile Glu Ala Gln Lys Thr Ile
565 570 575

Lys Lys Gln Ser Glu Gln Leu Lys Val Leu Gln Ala Ser Leu Glu Asp
580 585 590

Thr Gln Arg Gln Leu Gln Gln Thr Leu Asp Gln Tyr Ala Leu Ala Gln
595 600 605

Arg Lys Val Ser Ala Leu Ser Ala Glu Leu Glu Glu Cys Lys Val Ala
610 615 620

Leu Asp Asn Ala Ile Arg Ala Arg Lys Gln Ala Glu Ile Asp Leu Glu

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625	630	635	640
Glu Ala Asn Gly Arg Ile Thr Asp Leu Val Ser Ile Asn Asn Asn Leu			
	645	650	655
Thr Ala Ile Lys Asn Lys Leu Glu Thr Glu Leu Ser Thr Ala Gln Ala			
	660	665	670
Asp Leu Asp Glu Ala Thr Lys Glu Leu His Ala Ala Asp Glu Arg Ala			
	675	680	685
Asn Arg Ala Leu Ala Asp Ala Ala Arg Ala Val Glu Gln Leu His Glu			
	690	695	700
Glu Gln Glu His Ser Met Lys Ile Asp Ala Leu Arg Lys Ser Leu Glu			
705	710	715	720
Glu Gln Val Lys Gln Leu Gln Val Gln Ile Gln Glu Ala Glu Ala Ala			
	725	730	735
Ala Leu Leu Gly Gly Lys Arg Val Ile Ala Lys Leu Glu Thr Arg Ile			
	740	745	750
Arg Asp Leu Glu Thr Ala Leu Asp Glu Glu Thr Arg Arg His Lys Glu			
	755	760	765
Thr Gln Gly Ala Leu Arg Lys Lys Asp Arg Arg Ile Lys Glu Val Gln			
	770	775	780
Met Gln Val Asp Glu Glu His Lys Met Phe Val Met Ala Gln Asp Thr			
785	790	795	800
Ala Asp Arg Leu Leu Glu Lys Leu Asn Ile Gln Lys Arg Gln Leu Gly			
	805	810	815
Glu Ala Glu Ser Leu Thr Met Ala Asn Leu Gln Arg Val Arg Arg Tyr			
	820	825	830

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Gln Arg Glu Leu Glu Asp Ala Glu Asp Arg Ala Asp Thr Ala Glu Ser
835 840 845

Ser Leu Asn Ile Ile Arg Ala Lys His Arg Thr Phe Val Thr Thr Ser
850 855 860

Thr Val Pro Gly Ser Gln Val Tyr Ile Gln Glu Thr Thr Arg Thr Ile
865 870 875 880

Thr Glu Pro Pro Glu Arg Glu His Pro Arg Val
885 890

<210> 18

<211> 872

<212> PRT

<213> *Caenorhabditis elegans*

<400> 18

Met Ser Leu Tyr Arg Ser Pro Ser Ala Ala Leu Leu Lys Ser Pro Ser
1 5 10 15

Gln Ala Ala Phe Gly Ala Pro Phe Gly Ser Met Ser Val Ala Asp Leu
20 25 30

Gly Ser Leu Thr Arg Leu Glu Asp Lys Ile Arg Leu Leu Gln Glu Asp
35 40 45

Leu Glu Ser Glu Arg Glu Leu Arg Asn Arg Val Glu Arg Glu Arg Ala
50 55 60

Asp Leu Ser Val Gln Val Ile Ala Leu Thr Asp Arg Leu Glu Asp Ala
65 70 75 80

Glu Gly Thr Thr Asp Ser Gln Ile Glu Ser Asn Arg Lys Arg Glu Gly
85 90 95

Glu Leu Ser Lys Leu Arg Lys Leu Leu Glu Glu Ser Gln Leu Glu Ser

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100	105	110
Glu Asp Ala Met Asn Val Leu Arg Lys Lys His Gln Asp Ser Cys Leu		
115	120	125
Asp Tyr Gln Asp Gln Ile Glu Gln Leu Gln Lys Lys Asn Ala Lys Ile		
130	135	140
Asp Arg Glu Arg Gln Arg Val Gln His Glu Val Ile Glu Leu Thr Ala		
145	150	155 160
Thr Ile Asp Gln Leu Gln Lys Asp Lys His Thr Ala Glu Lys Ala Ala		
165	170	175
Glu Arg Phe Glu Ala Gln Ala Asn Glu Leu Ala Asn Lys Val Glu Asp		
180	185	190
Leu Asn Lys His Val Asn Asp Leu Ala Gln Gln Arg Gln Arg Leu Gln		
195	200	205
Ala Glu Asn Asn Asp Leu Leu Lys Glu Val His Asp Gln Lys Val Gln		
210	215	220
Leu Asp Asn Leu Gln His Val Lys Tyr Thr Leu Ala Gln Gln Leu Glu		
225	230	235 240
Glu Ala Arg Arg Arg Leu Glu Asp Ala Glu Arg Glu Arg Ser Gln Leu		
245	250	255
Gln Ser Gln Leu His Gln Val Gln Leu Glu Leu Asp Ser Val Arg Thr		
260	265	270
Ala Leu Asp Glu Glu Ser Ile Ala Arg Ser Asp Ala Glu His Lys Leu		
275	280	285
Asn Leu Ala Asn Thr Glu Ile Thr Gln Trp Lys Ser Lys Phe Asp Ala		
290	295	300

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Glu Val Ala Leu His His Glu Glu Val Glu Asp Leu Arg Lys Lys Met
305 310 315 320

Leu Gln Lys Gln Ala Glu Tyr Glu Glu Gln Ile Glu Ile Met Leu Gln
325 330 335

Lys Ile Ser Gln Leu Glu Lys Ala Lys Ser Arg Leu Gln Ser Glu Val
340 345 350

Glu Val Leu Ile Val Asp Leu Glu Lys Ala Gln Asn Thr Ile Ala Leu
355 360 365

Leu Glu Arg Ala Arg Glu Gln Leu Glu Arg Gln Val Gly Glu Leu Lys
370 375 380

Val Arg Ile Asp Glu Ile Thr Val Glu Leu Glu Ala Ala Gln Arg Glu
385 390 395 400

Leu Arg Ala Val Asn Ala Glu Leu Gln Lys Met Lys His Leu Tyr Glu
405 410 415

Lys Ala Val Glu Gln Lys Glu Ala Leu Ala Arg Glu Asn Lys Lys Leu
420 425 430

His Asp Glu Leu His Glu Ala Lys Glu Ala Leu Ala Asp Ala Asn Arg
435 440 445

Lys Leu His Glu Leu Asp Leu Glu Asn Ala Arg Leu Ala Gly Glu Ile
450 455 460

Arg Glu Leu Gln Thr Ala Leu Lys Glu Ala Asp Ala Gln Arg Arg Asp
465 470 475 480

Ala Glu Asn Arg Ala Gln Arg Ala Leu Ala Glu Leu Gln Ala Leu Arg
485 490 495

Ile Glu Met Glu Arg Arg Leu Gln Glu Lys Glu Glu Glu Met Glu Ala
500 505 510

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Leu Arg Lys Asn Leu Gln Phe Glu Ile Asp Arg Leu Ile Ala Ala Leu
515 520 525

Ala Asp Ala Glu Ala Arg Met Lys Ser Glu Ile Ser Arg Leu Lys Lys
530 535 540

Lys Tyr Gln Ala Glu Ile Ala Glu Leu Glu Met Thr Val Asp Asn Leu
545 550 555 560

Asn Arg Ala Asn Ile Glu Ala Gln Lys Thr Ile Lys Lys Gln Ser Glu
565 570 575

Gln Leu Lys Ile Leu Gln Ala Ser Leu Glu Asp Thr Gln Arg Gln Leu
580 585 590

Gln Gln Val Leu Asp Gln Tyr Ala Leu Ala Gln Arg Lys Val Ala Ala
595 600 605

Leu Ser Ala Glu Leu Glu Glu Cys Lys Thr Ala Leu Asp Asn Ala Ile
610 615 620

Arg Ala Arg Lys Gln Ala Glu Val Asp Leu Glu Glu Ala Asn Gly Arg
625 630 635 640

Ile Ser Asp Leu Ile Ser Ile Asn Asn Asn Leu Thr Ser Ile Lys Asn
645 650 655

Lys Leu Glu Thr Glu Leu Ser Thr Ala Gln Ala Asp Leu Asp Glu Val
660 665 670

Thr Lys Glu Leu His Ala Ala Asp Glu Arg Ala Asn Arg Ala Leu Ala
675 680 685

Asp Ala Ala Arg Ala Val Glu Gln Leu His Glu Glu Gln Glu His Ser
690 695 700

Met Lys Ile Asp Ala Leu Arg Lys Ser Leu Glu Glu Gln Val Lys Gln

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705	710	715	720
Leu Gln Val Gln Ile Gln Glu Ala Glu Ala Ala Ala Leu Leu Gly Gly			
725	730	735	
Lys Arg Val Ile Ala Lys Leu Glu Thr Arg Ile Arg Asp Leu Glu Thr			
740	745	750	
Ala Leu Asp Glu Glu Thr Arg Arg His Lys Glu Thr Gln Asn Ala Leu			
755	760	765	
Arg Lys Lys Asp Arg Arg Ile Lys Glu Val Gln Gln Leu Val Asp Glu			
770	775	780	
Glu His Lys Asn Phe Val Met Ala Gln Asp Thr Ala Asp Arg Leu Thr			
785	790	795	800
Glu Lys Leu Asn Ile Gln Lys Arg Gln Leu Ala Glu Ser Glu Ser Val			
805	810	815	
Thr Met Gln Asn Leu Gln Arg Val Arg Arg Tyr Gln His Glu Leu Glu			
820	825	830	
Asp Ala Glu Gly Arg Ala Asp Gln Ala Glu Ser Ser Leu His Leu Ile			
835	840	845	
Arg Ala Lys His Arg Ser Ser Val Val Thr Gly Lys Ser Ser Ser Lys			
850	855	860	
Ile Phe Val Thr Glu Asp Asp Tyr			
865	870		

<210> 19

<211> 20

<212> PRT

<213> Artificial Sequence

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<220>

<223> Description of Artificial Sequence:primer

<400> 19

Ala Ala Thr Thr Ala Ala Cys Cys Cys Thr Cys Ala Cys Thr Ala Ala

1

5

10

15

Ala Gly Gly Gly

20

<210> 20

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 20

Cys Gly Gly Gly Ala Thr Ala Thr Cys Ala Cys Thr Cys Ala Gly Cys

1

5

10

15

Ala Thr Ala Ala Thr Gly

20

<210> 21

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 21

Cys Thr Thr Cys Ala Thr Cys Cys Thr Cys Cys Ala Ala Thr Cys Gly

1

5

10

15

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Gly Thr Gly Thr

20

<210> 22

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 22

Gly Gly Thr Cys Gly Cys Thr Cys Ala Gly Ala Ala Gly Ala Cys Gly

1

5

10

15

Gly Thr Ala Cys

20

<210> 23

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 23

Ala Ala Gly Cys Ala Gly Thr Gly Gly Thr Ala Ala Cys Ala Ala Cys

1

5

10

15

Gly Cys Ala Gly Ala Gly Thr

20

<210> 24

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<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 24

Cys Gly Gly Gly Ala Thr Ala Thr Cys Ala Cys Thr Cys Ala Gly Cys
1 5 10 15

Ala Thr Ala Ala Thr Gly
20

<210> 25

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 25

Cys Gly Gly Gly Ala Thr Cys Cys Ala Thr Gly Gly Cys Gly Gly Cys
1 5 10 15

Thr Cys Gly Ala Thr Cys Ala Gly Cys Ala
20 25

<210> 26

<211> 29

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

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<400> 26

Cys Gly Cys Thr Cys Gly Ala Gly Thr Thr Ala Ala Ala Ala Gly Thr

1

5

10

15

Thr Cys Gly Ala Gly Cys Thr Cys Thr Cys Thr Thr Gly

20

25

<210> 27

<211> 3111

<212> DNA

<213> *Blomia tropicalis*

<400> 27

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cccaagtggc gtacactgca ttcgcaagtc tctcacatca tcttcaccag aagcagagta 180
gtagacgata aacgaaatca gtctcatcct acttctaaag cattgaacac ctttctacac 240
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tgatttgaaa caataatcaa cgacaatggc ggctcgatca gcaaagtata tgtatcagtc 360
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tgcttcatgg aaatccaaat acgaagccga acttcaagct cacgttgatg aggttgaaga 1260
acttcgacga aagatggccc agaagatttc ggaatatgag gaacaattgg aagcattggt 1320
gaacaaatgt agtgcattgg aaaagcaaaa ggctcgactc caatcggagg cagaagtttt 1380

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gatcatggac ttggaaaagg ctaccgcaca tgcccaggca ttggagaaac gcgtttccca 1440
attggagaag atcaacctcg acctcaagag caagttggag gaggtcagca tgttgttga 1500
acaaactcaa aaggatctcc gtgtcaagat tgcagacttg cagaaattgc aacacgagta 1560
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tttggctgaa gctaaatctc agttgaatga cgcccaccga cgaatccatg aacaagagat 1680
cgagatcaaa cgattggaga acgagcgaga agaattggct gccgcataca aggaagctga 1740
aaccttgcca aaacaagaag aggcaaagaa tcaacgattg actgccgagt tggcacagac 1800
acgacacgac tatgagaaac gattggcaca aaaggaagag gagattgaag ctcttcgaaa 1860
acaataccaa attgaaattg aacaattgaa catgcgattg gctgaggctg aagctaaatt 1920
gaagaccgag gttgcacgac tcaagaagaa gtaccaagca caaatcactg aattggaatt 1980
gtcgttggat gccgctaata aggctaacat cgacttgag aagacgatca agaagcaagc 2040
tctccaaatc actgagcttc aagcccatta tgatgagga catcgtcaat tgcaacaagc 2100
tggtgatcaa ttgggtgtca ctcaacgacg atgccaagca ttgaccgccg aattggagga 2160
gatgcgagtc aacttggaac aagcacttcg tgctaaacga gctgccgaac aaatgcacga 2220
agaggcagtt gtccgagtc aacgaacttac aaccatcaat gtcaacctcg cttcggccaa 2280
gagcaaattg gagactgaat tctctgctct tcaaaacgat tacgatgaag tacacaagga 2340
actccgaatc tctgacgaac gagtgcacaa acttaccatt gaggtcaagt cgacaaagga 2400
cttgctcgaa tcagaaacgg aacgtgtcac aaaattggaa acaatcaaga aatcattgga 2460
aaccgaagta cgaaatctcc aaatccgaat tgaagaagtt gaggctaatt cattggctgg 2520
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gtccgataaa ttgaacgaaa aggtcaaggt ttacaaacga caaatgcaag aacaggaggg 2760
aatgagccaa caaaacttga cccgtgtccg acgattccaa cgagagttgg aagccgccga 2820
agaccgagct gatcaagctg aatccaattt gtcgttcac cgagccaaac atcgatcgtg 2880
ggtcaccacc agtcaagtcc ctggcggtac tcgacaagtc tttgtcacgg aacaagagag 2940
ctcgaacttt taaataacag catgcaacaa attcattatt tcatataaat cgaacaataa 3000
tttgaaattc aaaccaaaca tagatataaa tctaataaaa aaaaagtfff aaaaaaatca 3060
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<210> 28

<211> 1014

<212> DNA

<213> *Blomia tropicalis*

<220>

<221> CDS

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<222> (7)..(858)

<400> 28

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1 5 10

aag gat aat gcc atc gat cga gct gag att gcc gaa caa aag tcg cga 96
Lys Asp Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ser Arg
15 20 25 30

gat gcc aac ctc aga gcc gag aag tcc gaa gag gaa gtc cgt gcc ctt 144
Asp Ala Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Ala Leu
35 40 45

caa aag aag att caa caa att gaa aat gaa ttg gat caa gtc caa gaa 192
Gln Lys Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu
50 55 60

tcg ttg acc caa gcc aat acc aaa ttg gaa gag aag gaa aag tca ctc 240
Ser Leu Thr Gln Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ser Leu
65 70 75

caa acg gcc gaa ggt gat gtc gct gct ctt aac cga cgt atc caa ctc 288
Gln Thr Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu
80 85 90

att gaa gag gat ttg gaa cga tcc gaa gag cga ctc aag gtt gcc acc 336
Ile Glu Glu Asp Leu Glu Arg Ser Glu Glu Arg Leu Lys Val Ala Thr
95 100 105 110

gcc aag ctc gaa gag gct tca cac tct gcc gat gaa tct gaa cgt atg 384
Ala Lys Leu Glu Glu Ala Ser His Ser Ala Asp Glu Ser Glu Arg Met
115 120 125

cgt aag atg ctt gaa cat cga agc atc acc gat gag gaa cgt atg gat 432
Arg Lys Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Asp
130 135 140

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ggc ctc gag agt caa tta aaa gaa gcc cgt atg atg gct gaa gat gct 480
Gly Leu Glu Ser Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala
145 150 155

gac cga aaa tat gat gaa gtt gcc cgt aaa ttg gcc atg gtt gag gct 528
Asp Arg Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala
160 165 170

gat ctt gaa cgt gct gaa gag cga gcc gaa acc ggt gaa aca aag att 576
Asp Leu Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Thr Lys Ile
175 180 185 190

gtt gaa ctc gaa gaa gag ttg cga gtc gtt ggt aac aac ttg aaa tcg 624
Val Glu Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser
195 200 205

ctc gaa gtc agc gaa gag aag gcc caa caa cgt gag gaa gct tat gag 672
Leu Glu Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala Tyr Glu
210 215 220

caa caa atc cga atg atg acc ggc aag ctc aag gag gct gag gcc cga 720
Gln Gln Ile Arg Met Met Thr Gly Lys Leu Lys Glu Ala Glu Ala Arg
225 230 235

gct gaa ttt gca gaa cga tcg gtt caa aag ttg caa aag gaa gtc gat 768
Ala Glu Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp
240 245 250

cga ctg gaa gac gag ttg gtg cac gag aag gaa aaa tac aaa tca atc 816
Arg Leu Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile
255 260 265 270

tcg gac gaa ttg gac cag aca ttt gcc gaa ctc act ggt tat 858
Ser Asp Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr
275 280

taagcttata tacgccatcg atcgacgccg acccaacagc caaaaacaac caagttgtca 918

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taaaatccaa tgctctgtgc actgtttccg cttatatatt ttatttatta ttaaatacatt 978

caaataatat aatgatggag acgaatgcaa atcaaa

1014

<210> 29

<211> 284

<212> PRT

<213> *Blomia tropicalis*

<400> 29

Met Glu Ala Ile Lys Lys Lys Met Gln Ala Met Lys Leu Glu Lys Asp

1

5

10

15

Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ser Arg Asp Ala

20

25

30

Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Ala Leu Gln Lys

35

40

45

Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Ser Leu

50

55

60

Thr Gln Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ser Leu Gln Thr

65

70

75

80

Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu

85

90

95

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

100

105

110

Leu Glu Glu Ala Ser His Ser Ala Asp Glu Ser Glu Arg Met Arg Lys

115

120

125

Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Asp Gly Leu

130

135

140

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Glu Ser Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala Asp Arg
145 150 155 160

Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala Asp Leu
165 170 175

Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Thr Lys Ile Val Glu
180 185 190

Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser Leu Glu
195 200 205

Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala Tyr Glu Gln Gln
210 215 220

Ile Arg Met Met Thr Gly Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu
225 230 235 240

Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp Arg Leu
245 250 255

Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp
260 265 270

Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr
275 280

<210> 30

<211> 852

<212> DNA

<213> Blomia tropicalis

<220>

<221> CDS

<222> (1)..(852)

- 53 -

<400> 30

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 1 5 10 15

aat gcc atc gat cga gct gag att gcc gaa caa aag tcg cga gat gcc 96
 Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ser Arg Asp Ala
 20 25 30

aac ctc aga gcc gag aag tcc gaa gag gaa gtc cgt gcc ctt caa aag 144
 Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Ala Leu Gln Lys
 35 40 45

aag att caa caa att gaa aat gaa ttg gat caa gtc caa gaa tcg ttg 192
 Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Ser Leu
 50 55 60

acc caa gcc aat acc aaa ttg gaa gag aag gaa aag tca ctc caa acg 240
 Thr Gln Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ser Leu Gln Thr
 65 70 75 80

gcc gaa ggt gat gtc gct gct ctt aac cga cgt atc caa ctc att gaa 288
 Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu
 85 90 95

gag gat ttg gaa cga tcc gaa gag cga ctc aag gtt gcc acc gcc aag 336
 Glu Asp Leu Glu Arg Ser Glu Glu Arg Leu Lys Val Ala Thr Ala Lys
 100 105 110

ctc gaa gag gct tca cac tct gcc gat gaa tct gaa cgt atg cgt aag 384
 Leu Glu Glu Ala Ser His Ser Ala Asp Glu Ser Glu Arg Met Arg Lys
 115 120 125

atg ctt gaa cat cga agc atc acc gat gag gaa cgt atg gat ggc ctc 432
 Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Asp Gly Leu
 130 135 140

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gag agt caa tta aaa gaa gcc cgt atg atg gct gaa gat gct gac cga	480
Glu Ser Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala Asp Arg	
145 150 155 160	
aaa tat gat gaa gtt gcc cgt aaa ttg gcc atg gtt gag gct gat ctt	528
Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala Asp Leu	
165 170 175	
gaa cgt gct gaa gag cga gcc gaa acc ggt gaa aca aag att gtt gaa	576
Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Thr Lys Ile Val Glu	
180 185 190	
ctc gaa gaa gag ttg cga gtc gtt ggt aac aac ttg aaa tcg ctc gaa	624
Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser Leu Glu	
195 200 205	
gtc agc gaa gag aag gcc caa caa cgt gag gaa gct tat gag caa caa	672
Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala Tyr Glu Gln Gln	
210 215 220	
atc cga atg atg acc ggc aag ctc aag gag gct gag gcc cga gct gaa	720
Ile Arg Met Met Thr Gly Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu	
225 230 235 240	
ttt gca gaa cga tcg gtt caa aag ttg caa aag gaa gtc gat cga ctg	768
Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp Arg Leu	
245 250 255	
gaa gac gag ttg gtg cac gag aag gaa aaa tac aaa tca atc tcg gac	816
Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp	
260 265 270	
gaa ttg gac cag aca ttt gcc gaa ctc act ggt tat	852
Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr	
275 280	

<210> 31

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<211> 284

<212> PRT

<213> *Blomia tropicalis*

<400> 31

Met Glu Ala Ile Lys Lys Lys Met Gln Ala Met Lys Leu Glu Lys Asp

1

5

10

15

Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ser Arg Asp Ala

20

25

30

Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Ala Leu Gln Lys

35

40

45

Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Ser Leu

50

55

60

Thr Gln Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ser Leu Gln Thr

65

70

75

80

Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu

85

90

95

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

100

105

110

Leu Glu Glu Ala Ser His Ser Ala Asp Glu Ser Glu Arg Met Arg Lys

115

120

125

Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Asp Gly Leu

130

135

140

Glu Ser Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala Asp Arg

145

150

155

160

Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala Asp Leu

165

170

175

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Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Thr Lys Ile Val Glu
180 185 190

Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser Leu Glu
195 200 205

Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala Tyr Glu Gln Gln
210 215 220

Ile Arg Met Met Thr Gly Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu
225 230 235 240

Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp Arg Leu
245 250 255

Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp
260 265 270

Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr
275 280

<210> 32

<211> 284

<212> PRT

<213> Dermatophagoides farinae

<400> 32

Met Glu Ala Ile Lys Lys Lys Met Gln Ala Met Lys Leu Glu Lys Asp
1 5 10 15

Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ala Arg Asp Ala
20 25 30

Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Ala Leu Gln Lys
35 40 45

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Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Gln Leu
50 55 60

Ser Ala Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ala Leu Gln Thr
65 70 75 80

Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu
85 90 95

Glu Asp Leu Glu Arg Ser Glu Glu Arg Leu Lys Ile Ala Thr Ala Lys
100 105 110

Leu Glu Glu Ala Ser Gln Ser Ala Asp Glu Ser Glu Arg Met Arg Lys
115 120 125

Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Asp Gly Leu
130 135 140

Glu Asn Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala Asp Arg
145 150 155 160

Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala Asp Leu
165 170 175

Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Ser Lys Ile Val Glu
180 185 190

Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser Leu Glu
195 200 205

Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala Tyr Glu Gln Gln
210 215 220

Ile Arg Ile Met Thr Ala Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu
225 230 235 240

Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp Arg Leu
245 250 255

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Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp
260 265 270

Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr
275 280

<210> 33

<211> 284

<212> PRT

<213> *Lepidoglyphus destructor*

<400> 33

Met Glu Ala Ile Lys Asn Lys Met Gln Ala Met Lys Leu Glu Lys Asp
1 5 10 15

Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ser Arg Asp Ala
20 25 30

Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Gly Leu Gln Lys
35 40 45

Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Ser Leu
50 55 60

Thr Gln Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ser Leu Gln Thr
65 70 75 80

Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu
85 90 95

Glu Asp Leu Glu Arg Ser Glu Gly Arg Leu Lys Ile Ala Thr Ser Lys
100 105 110

Leu Glu Glu Ala Ser Gln Ser Ala Asp Glu Ser Glu Arg Met Arg Lys
115 120 125

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Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Glu Gly Leu

130

135

140

Glu Ser Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala Asp Arg

145

150

155

160

Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala Asp Leu

165

170

175

Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Ser Lys Ile Val Glu

180

185

190

Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser Leu Glu

195

200

205

Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala Tyr Glu Gln Gln

210

215

220

Ile Arg Ile Met Thr Thr Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu

225

230

235

240

Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp Arg Leu

245

250

255

Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp

260

265

270

Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr

275

280

<210> 34

<211> 284

<212> PRT

<213> Dermatophagoides pteronyssinus

<400> 34

Met Glu Ala Ile Lys Lys Lys Met Gln Ala Met Lys Leu Glu Lys Asp

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

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Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala His Glu Gln Gln

210

215

220

Ile Arg Ile Met Thr Thr Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu

225

230

235

240

Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Asp Arg Leu

245

250

255

Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp

260

265

270

Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr

275

280

<210> 35

<211> 284

<212> PRT

<213> Dermatophagoides pteronyssinus

<400> 35

Met Glu Ala Ile Lys Asn Lys Met Gln Ala Met Lys Leu Glu Lys Asp

1

5

10

15

Asn Ala Ile Asp Arg Ala Glu Ile Ala Glu Gln Lys Ala Arg Asp Ala

20

25

30

Asn Leu Arg Ala Glu Lys Ser Glu Glu Glu Val Arg Ala Leu Gln Lys

35

40

45

Lys Ile Gln Gln Ile Glu Asn Glu Leu Asp Gln Val Gln Glu Gln Leu

50

55

60

Ser Ala Ala Asn Thr Lys Leu Glu Glu Lys Glu Lys Ala Leu Gln Thr

65

70

75

80

Ala Glu Gly Asp Val Ala Ala Leu Asn Arg Arg Ile Gln Leu Ile Glu

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85	90	95
Glu Asp Leu Glu Arg Ser Glu Glu Arg Leu Lys Ile Ala Thr Ala Lys		
100	105	110
Leu Glu Glu Ala Ser Gln Ser Ala Asp Glu Ser Glu Arg Met Arg Lys		
115	120	125
Met Leu Glu His Arg Ser Ile Thr Asp Glu Glu Arg Met Glu Gly Leu		
130	135	140
Glu Asn Gln Leu Lys Glu Ala Arg Met Met Ala Glu Asp Ala Asp Arg		
145	150	155 160
Lys Tyr Asp Glu Val Ala Arg Lys Leu Ala Met Val Glu Ala Asp Leu		
165	170	175
Glu Arg Ala Glu Glu Arg Ala Glu Thr Gly Glu Ser Lys Ile Val Glu		
180	185	190
Leu Glu Glu Glu Leu Arg Val Val Gly Asn Asn Leu Lys Ser Leu Glu		
195	200	205
Val Ser Glu Glu Lys Ala Gln Gln Arg Glu Glu Ala His Glu Gln Gln		
210	215	220
Ile Arg Ile Met Thr Thr Lys Leu Lys Glu Ala Glu Ala Arg Ala Glu		
225	230	235 240
Phe Ala Glu Arg Ser Val Gln Lys Leu Gln Lys Glu Val Gly Arg Leu		
245	250	255
Glu Asp Glu Leu Val His Glu Lys Glu Lys Tyr Lys Ser Ile Ser Asp		
260	265	270
Glu Leu Asp Gln Thr Phe Ala Glu Leu Thr Gly Tyr		
275	280	

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<210> 36

<211> 408

<212> DNA

<213> *Blomia tropicalis*

<400> 36

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acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttcggtttt ggctcaagag 60
cacaagccaa agaaggatga tttccgaaac gaattcgatc acttgttgat cgaacaggca 120
aaccatgcta tcgaaaaggg agaacatcaa ttgctttact tgcaacacca actcgacgaa 180
ttgaatgaaa acaagagcaa ggaattgcaa gagaaaatca ttcgagaact tgatgttggt 240
tgcgccatga tcgaaggagc ccaaggagct ttggaacgtg aattgaagcg aactgatctt 300
aacatttttg aacgattcaa ctacgaagag gctcaaactc tcagcaagat cttgcttaag 360
gatttgaagg aaaccgaaca aaaagtgaag gatattcaaa cccaataa 408
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<210> 37

<211> 117

<212> PRT

<213> *Blomia tropicalis*

<400> 37

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Gln Glu His Lys Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp His
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Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His Gln
20 25 30
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Leu Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser
35 40 45
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```
Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala
50 55 60
```

```
Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr
65 70 75 80
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```
Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu
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85

90

95

Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys

100

105

110

Asp Ile Gln Thr Gln

115

<210> 38

<211> 464

<212> DNA

<213> *Blomia tropicalis*

<400> 38

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aacagttttt tttttgaaag gttaataaaa aagtaatcaa agaaactttt taggcaaacc 180
atgctatcga aaagggagaa catcaattgc tttacttgca acaccaactc gacgaattga 240
atgaaaacaa gagcaaggaa ttgcaagaga aaatcattcg agaacttgat gttgtttgcg 300
ccatgatcga aggagcccaa ggagctttgg aacgtgaatt gaagcgaact gatcttaaca 360
ttttggaacg attcaactac gaagaggctc aaactctcag caagatcttg cttaaggatt 420
tgaaggaaac cgaacaaaaa gtgaaggata ttcaaacca ataa 464

<210> 39

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 39

acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttcggtttt ggctcaagag 60
cacaagccaa agaaggatga tttccgaaac gaattcgatc acttggtgat cgaacaggta 120
aacagttttt tttgaaagggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
gctatcgaaa aggggtgaaca tcaattgctt tacttgcaac accaactcga cgaattgaat 240
gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgcgcc 300
atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tcttaacatt 360
ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420

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aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa

462

<210> 40

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 40

acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttcggtttt ggctcaagag 60
 cacaagccaa agaaggatga tttccgaaac gaattcgatc acttggtgat cgaacaggta 120
 aacagttttt tttgaaaggt taataaaaaa gtaataaaaag aaacttttta ggcaaaccat 180
 gctatcgaaa aggggtgaaca tcaattgatt tacttgcaac accaactoga cgaattgaat 240
 gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgcgcc 300
 atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tottaacatt 360
 ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
 aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa 462

<210> 41

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 41

acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttcggtttt ggctcaagag 60
 cacaagccaa agaaggatga tttccgaaac gaattcgatc actggttgat cgaacaggta 120
 aacagttttt tttgaaaggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
 gctatcgaaa agggagaaca tcaattgctt tacttgcaac accaactoga cgaattgaat 240
 gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt cgtttgcgcc 300
 atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tottaacatt 360
 ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
 aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa 462

<210> 42

<211> 462

<212> DNA

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<213> *Blomia tropicalis*

<400> 42

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acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttecggtttt ggctcaagag 60
cacgagccaa agaaggatga tttccgaaac gaattcgatc acttgttgat cgaacaggta 120
aacagttttt tttgaaaggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
gctatcgaaa agggatgaaca tcaattgatt tacttgcaac accaactcga cgaattgaat 240
gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgccgc 300
atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tcttaacatt 360
ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa 462
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<210> 43

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 43

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acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttecggtttt ggctcaagag 60
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aacagttttt tttgaaaggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
gctatcgaaa agggagaaca tcaattgctt tacttgcaac accaactcga cgaattgaat 240
gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgccgc 300
atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tcttaacatt 360
ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
aaggaaaccg aacaaaaagt gaaggatatt caaaccaaat aa 462
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<210> 44

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 44

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acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttecggtttt ggctcaagag 60
cacaagccaa agaaggatga tttccgaaac gaattcgatc acttgttgat cgaacaggta 120
aacagttttt tttgaaaggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
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gctatcgaaa agggagaaca tcaattgctt tacttgcaac accaactcga cgaattgaat 240
gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgcgcc 300
atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tcttaacatt 360
ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa 462

<210> 45

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 45

acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttcggtttt ggctcaagag 60
cacaagccaa agaaggatga tttccgaaac gaattcgatc acttgttgat cgaacaggta 120
aacagttttt ttgaaaggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
gctatcgaaa aggggtgaaca tcaattgctt tacttgcaac accaactcga cgaattgaat 240
gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgcgcc 300
atgatcgaag gagcccaagg agctttggaa cgtgaattga agcgaactga tcttaacatt 360
ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa 462

<210> 46

<211> 462

<212> DNA

<213> *Blomia tropicalis*

<400> 46

acaatgaagt tcgccatcgt tcttattgcc tgctttgccg cttcggtttt ggctcaagag 60
cacaagccaa agaaggatga tttccgaaac gaattcgatc acttgttgat cgaacaggta 120
aacagttttt ttgaaaggt taataaaaaa gtaatcaaag aaacttttta ggcaaaccat 180
gctatcgaaa agggagaaca tcaattgctt cacttgcaac accaactcga cgaattgaat 240
gaaaacaaga gcaaggaatt gcaagagaaa atcattcgag aacttgatgt tgtttgcgcc 300
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ttggaacgat tcaactacga agaggctcaa actctcagca agatcttgct taaggatttg 420
aaggaaaccg aacaaaaagt gaaggatatt caaacccaat aa 462

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<210> 47

<211> 463

<212> DNA

<213> *Blomia tropicalis*

<400> 47

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aacagttttt ttttgaaagg ttaataaaaa agtaatcaaa gaaacttttt aggcaaacca 180
tgctatcgaa aagggagAAC atcaattgct ttacttgcaa caccaactcg acgaattgaa 240
tgaaaacaag agcaaggaat tgcaagagaa aatcattcga gaacttgatg ttgtttgcgc 300
catgatcgaa ggagcccaag gagctttgga acgtgaattg aagcgaactg atcttaacat 360
tttggAACga ttcaactacg aagaggctca aactctcagc aagatcttgc ttaaggattt 420
gaaggaaacc gaacaaaaag tgaaggatat tcaaaccCAA taa 463
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<210> 48

<211> 408

<212> DNA

<213> *Blomia tropicalis*

<400> 48

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caacagcaca agccaaagaa ggatgatttc cgaaacgaat tcgatcactt gttgatcgaa 60
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aaccatgcta tcgaaaaggg tgaacatcaa ttgctttact tgcaacacca actcgacgaa 180
ttgaatgaaa acaagagcaa ggaattgcaa gagaaaatca ttcgagaact tgatgttggt 240
tgcgccatga tcgaaggagc ccaaggagct ttggaacgtg aattgaagcg aactgatctt 300
aacatttttg aacgattcaa ctacgaagag gctcaaactc tcagcaagat cttgcttaag 360
gatttgaagg aaaccgaaca aaaagtgaag gatattcaaa cccaataa 408
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<210> 49

<211> 117

<212> PRT

<213> *Blomia tropicalis*

<400> 49

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Gln Glu His Lys Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp His
1 5 10 15

Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His Gln
20 25 30

Leu Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser
35 40 45

Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala
50 55 60

Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr
65 70 75 80

Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu
85 90 95

Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys
100 105 110

Asp Ile Gln Thr Gln
115

<210> 50

<211> 117

<212> PRT

<213> *Blomia tropicalis*

<400> 50

Gln Glu His Lys Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp His
1 5 10 15

Trp Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His Gln
20 25 30

Leu Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser

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35	40	45
Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala		
50	55	60
Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr		
65	70	75 80
Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu		
85	90	95
Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys		
100	105	110
Asp Ile Gln Thr Gln		
115		

<210> 51
 <211> 117
 <212> PRT
 <213> Blomia tropicalis

<400> 51

Gln Glu His Glu Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp His
1 5 10 15
Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His Gln
20 25 30
Ile Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser
35 40 45
Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala
50 55 60
Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr
65 70 75 80

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Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu
85 90 95

Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys
100 105 110

Asp Ile Gln Thr Gln
115

<210> 52

<211> 117

<212> PRT

<213> *Blomia tropicalis*

<400> 52

Gln Glu His Lys Pro Lys Lys Asp Asp Phe Arg Asp Glu Phe Asp His
1 5 10 15

Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His Gln
20 25 30

Leu His Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser
35 40 45

Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala
50 55 60

Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr
65 70 75 80

Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu
85 90 95

Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys
100 105 110

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Asp Ile Gln Thr Lys

115

<210> 53

<211> 117

<212> PRT

<213> *Blomia tropicalis*

<400> 53

Gln Gln His Lys Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp His

1

5

10

15

Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His Gln

20

25

30

Leu Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser

35

40

45

Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala

50

55

60

Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr

65

70

75

80

Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu

85

90

95

Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys

100

105

110

Asp Ile Gln Thr Gln

115

<210> 54

<211> 17

<212> PRT

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<213> Blomia tropicalis

<400> 54

Met Lys Phe Ala Ile Val Leu Ile Ala Cys Phe Ala Ala Ser Val Leu

1

5

10

15

Ala

<210> 55

<211> 930

<212> DNA

<213> Blomia tropicalis

<400> 55

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aaaaaaaaaga aagggataaa gaanaacaaa aaacaaatga caagtcctaa gtaaacaact 120
ttttaagtta aataaaaaag aaatagccag aactgaccta ccgnggccta acctagtata 180
catataaaca cagtaaaccct ctgtaaaaaa acatataaaa aacatcataa ctcagtcaaa 240
aaaagacaaa aaagacaaac caccgacaat actaacctca gagtaaaaag acataaaagg 300
gggaaccctt aaattttaaag aactactaag aaaccaaaac aaacaaaaaa caggactaga 360
aagtaacaca cccttttaaaa ccagcatact acgacaatga aaaatatcat aacaagatgt 420
tatcaggtaa aatatcaaag gtgagataaa accgtgggca actcggtttt ggctcaagag 480
cacaagccaa agaaggatga tttccgaaac gaattcgatc acttggttgat cgaacaggca 540
aaccatgcta tcgaaaaggg agaacatcaa ttgctttact tgcaacacca actcgacgaa 600
ttgaatgaaa acaagagcaa ggaattgcaa gagaaaaatca ttcgagaact tgatgttggt 660
tgcgccatga tcgaaggagc ccaaggagct ttggaacgtg aattgaagcg aactgatctt 720
aacatttttg aacgattcaa ctacgaagag gtcaaaactc tcagcaagat cttgcttaag 780
gatttgaagg aaaccgaaca aaaagtgaag gatattcaaa cccaataaaa atttagaatt 840
gtacaatttt acatttttga tatgattaaa tgtcaataaa tgttcaataa ataaattcaa 900
tttttaacta taaaaaaaaa aaaaaaaaaa 930
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<210> 56

<211> 899

<212> DNA

<213> Blomia tropicalis

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<400> 56

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caaagataat tagactcgtt tgtaaacgcg caggcaactg acattggtgc agattgaggt 180
cactttgatt tcatctccag taaactctcg aatgtatttg atctccaaat ctttgtcttt 240
ttggatttgg gtataggtgt tgccctcagc agtaacaata gacttgacct tttttccatc 300
cattcgttct tcatcaaatt cttcaccaag cttgaatttg gttgtttgtg ttttgaaggg 360
agcggtagcc ttgggttagg aaaacactca caatccacaa actcaaacaa caatgaagtt 420
cgccategtt cttattgcct gctttgccgc ttcggttttg gctcaagagc acaagccaaa 480
gaaggatgat ttccgaaacg aattcgatca cttgttgatc gaacaggcaa accatgctat 540
cgaaaaggga gaacatcaat tgctttactt gcaacaccaa ctcgacgaat tgaatgaaaa 600
caagagcaag gaattgcaag agaaaatcat tctagaactt gatgttgttt gcgccatgat 660
cgaaggagcc caaggagctt tggaacgtga attgaagcga actgatctta acattttgga 720
acgattcaac tacgaagagg ctcaaactct cagcaagatc ttgcttaagg atttgaagga 780
aaccgaacaa aaagtgaagg atattcaaac ccaataaaaa tttagaattg tacaatttta 840
catttttgat atgattaaat gtcaataaat gttcaataaa taaaaaaaaa aaaaaaaaaa 899
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<210> 57

<211> 923

<212> DNA

<213> *Blomia tropicalis*

<400> 57

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gtcgtgcgct gcacactaaa tgtttggcaa ctcaaaacta tacacaattt ggcaactagt 60
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tcggctgctg gttgcagtat gatctatacc gaacctagca caatggccgc cggttcagca 180
tatatatata tatatttaaa gtaaaataat tatatatatg taaaacaaat taaaacccta 240
tgatgataat atatgtattg tacgtgtgta tacacttggtg tacggataaa tgggtgaaaac 300
aatgtgtcat ccggtcaatg tgtatgtttt tggaaagata tctgtttttt tgatgcgctt 360
gtttcgatgt ctaaaacact cacaatccac aaactcaaac aacaatgaag ttcgccatcg 420
ttcttattgc ctgctttgcc gcttcggttt tggctcaaga gcacaagcca aagaaggatg 480
atttccgaaa cgaattcgat cacttggtga tcgaacaggc aaacctgct atcgaaaagg 540
gagaacatca attgctttac ttgcaacacc aactcgacga attgaatgaa aacaagagca 600
aggaattgca agagaaaatc attcgagaac ttgatgttgt ttgcgccatg atcgaaggag 660
cccaaggagc tttggaacgt gaattgaagc gaactgatct taacattttg gaacgattca 720
actacgaaga ggctcaaact ctgagcaaga tcttgcttaa ggatttgaag gaaaccgaac 780
```

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aaaaagtgaa ggatattcaa acccaataaa aatttagaat tgtacaattt tacatttttg 840
 atatgattaa atgtcaataa atgttcaata aataaattca atttttaact ataaaaaaaa 900
 aaaaaaaaaa aaaaaaaaaa aaa 923

<210> 58

<211> 892

<212> DNA

<213> *Blomia tropicalis*

<400> 58

taaacgaaaa tatggtcattg tttattcatt atcaactcaa aagtttctat tatctacaaa 60
 gtcaaattta ttatatgtta ttttttcaaa gtgatcgga tctccttgtc ggcctttgcc 120
 tcaatttggtg gcaatgggtgc ttcaatcacc aatctaccat cggttttcaa cgatgaacgg 180
 actttgtcca aatcggcac ctcaggcaac gagtaacgat agttaagac cgggttttgt 240
 ttaagattag aatcggttatg ttcaactcttg gcattgatga ttatttcacc tttcgtaacg 300
 gtcacttgaa tatcttccgg cttaaagtct ggcaaatcga tttcaatctt aaagattcgt 360
 tgtccacaca aactcaaaca acaatgaagt tcgccatcgt tcttattgcc tgctttgccg 420
 cttcggtttt ggctcaagag cacaagccaa agaaggatga tttccgaaac gaattcgatc 480
 acttggtgat cgaacaggca aaccatgcta tcgaaaaggg agaacatcaa ttgctttact 540
 tgcaacacca actcgacgaa ttgaatgaaa acaagagcaa ggaattgcaa gagaaaatca 600
 ttcgagaact tgatgttggt tgcgccatga tcgaaggagc ccaaggagct ttggaacgtg 660
 aattgaagcg aactgatctt aacatttttg aacgattcaa ctacgaagag gctcaaactc 720
 tcagcaagat cttgcttaag gatttgaagg aaaccgaaca aaaagtgaag gatattcaaa 780
 cccaataaaa atttagaatt gtacaatttt acatttttga tatgattaaa tgtcaataaa 840
 tgttcaataa ataaattcaa tttttaaaaa aaaaaaaaaa aaaaaaaaaa aa 892

<210> 59

<211> 738

<212> DNA

<213> *Blomia tropicalis*

<400> 59

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 cncattttat ttcgattgat tgggcaaata gagtcattta catcgatttg ggtttggggg 120
 ggcatacaaa ctgaacgatg acgagcccga ccaggacgac gacgacgttt atcgttgtcc 180
 atacgggttg tttgttttagg aaaacactca caatccacaa actcaaacaa caatgaagtt 240

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```
cgccatcggtt cttattgcct gctttgccgc ttcggttttg gctcaagagc acaagccaaa 300
gaaggatgat ttccgaaacg aattcgatca cttgttgatc gaacaggcaa accatgctat 360
cgaaaagggga gaacatcaat tgctttactt gcaacaccaa ctcgacgaat tgaatgaaaa 420
caagagcaag gaattgcaag agaaaatcat tcgagaactt gatgttggtt gcgccatgat 480
cgaaggagcc caaggagctt tggaacgtga attgaagcga actgatctta acattttgga 540
acgattcaac tacgaagagg ctcaaactct cagcaagatc ttgcttaagg atttgaagga 600
aaccgaacaa aaagtgaagg atattcaaac ccaataaaaa tttagaattg tacaatttta 660
catTTTTgat atgattaaat gtcaataaat gttcaataaa taaattcaat ttttaactat 720
aaaaaaaaaa aaaaaaaaaa 738
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<210> 60

<211> 557

<212> DNA

<213> *Blomia tropicalis*

<400> 60

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tccttgttta ggaaaacact ctctatccac aaactcaaac aacaatgaag ttcgccatcg 60
ttcttattgc ctgctttgcc gcttcggttt tggctcaaga gcacaagcca aagaaggatg 120
atttccgaaa cgaattcgat cacttggttg tcgaacaggc aaaccatgct atcgaaaagg 180
gagaacatca attgctttac ttgcaacacc aactcgacga attgaatgaa aacaagagca 240
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cccaaggagc tttggaacgt gaattgaagc gaactgatct taacattttg gaacgattca 360
actacgaaga ggctcaaact ctgagcaaga tcttgcttaa ggatttgaag gaaaccgaac 420
aaaaagtga ggaatttcaa acccaataaa aatttagaat tgtacaattt tacatttttg 480
atatgattaa atgtcaataa atgttcaata aataaattca atttttaaaa aaaaaaaaaa 540
aaaaaaaaaa aaaaaaa 557
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<210> 61

<211> 520

<212> DNA

<213> *Blomia tropicalis*

<400> 61

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aaaacactca caatccacaa actcaaacaa caatgaagtt cgccatcggt cttattgcct 60
gctttgccgc ttcggttttg gctcaagagc acaagccaaa gaaggatgat ttccgaaacg 120
aattcgatca cttgttgatc gaacaggcaa accatgctat cgaaaagggga gaacatcaat 180
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tgctttactt gcaacaccaa ctcgacgaat tgaatgaaaa caagagcaag gaattgcaag 240
agaaaatcat tcgagaactt gatgttggtt ggcgccatgat cgaaggagcc caaggagctt 300
tggaacgtga attgaagcga actgatctta acattttgga acgattcaac tacgaagagg 360
ctcaaactct cagcaagatc ttgcttaagg atttgaagga aaccgaacaa aaagtgaagg 420
atattcaaac ccaataaaaa tttagaattg tacaatttta catttttgat atgattaaat 480
gtcaataaat gttcaataaa taaaaaaaaa aaaaaaaaaa 520

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<210> 62

<211> 523

<212> DNA

<213> *Blomia tropicalis*

<400> 62

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ttaggaaaac actcacaatc cacaaactca aacaacaatg aagttcgcca tcgttcttat 60
tgcttgcttt gccgcttcgg ttttggtcga agagcacaag ccaaagaagg atgatttccg 120
aaacgaattc gatcacttgt tgatcgaaca ggcaaaccat gctatcgaaa agggagaaca 180
tcaattgctt tacttgcaac accaactcga cgaattgaat gaaaacaaga gcaaggaatt 240
gcaagagaaa atcattcgag aacttgatgt tgtttgccc atgatcgaag gagcccaagg 300
agctttggaa cgtgaattga agcgaactga tcttaacatt ttggaacgat tcaactacga 360
agaggctcaa actctcagca agatcttgct taaggatttg aaggaaaccg aacaaaaagt 420
gaaggatatt caaacccaat aaaaatttag aattgtacaa ttttacattt ttgatatgat 480
taaagtgtcaa taaatgttca ataaaaaaaa aaaaaaaaaa aaa 523

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<210> 63

<211> 521

<212> DNA

<213> *Blomia tropicalis*

<400> 63

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aaaacactca caatccacaa actcaaacaa caatgaagtt cgccatcggt cttattgcct 60
gctttgccc ttcggttttg gctcaagagc acaagccaaa gaaggatgat ttccgaaacg 120
aattcgatca cttgttgatc gaacaggcaa accatgctat cgaaaatgga gaacatcaat 180
tgctttactt gcaacaccaa ctcgacgaat tgaatgaaaa caagagcaag gaattgcaag 240
agaaaatcat tcgagaactt gatgttggtt ggcgccatgat cgaaggagcc caaggagctt 300
tggaacgtga attgaagcga actgatctta acattttgga acgattcaac tacgaagagg 360
ctcaaactct cagcaagatc ttgcttaagg atttgaagga aaccgaacaa aaagtgaagg 420

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atattcaaac ccaataaaaa tttagaattg tacaatttta catttttgat atgattaaat 480
gtcaataaat gttcaataaa taaattcaat ttttaactat a 521

<210> 64

<211> 509

<212> DNA

<213> *Blomia tropicalis*

<400> 64

tgcttgcttt gccgcttcgg ttttggtca agagcacaag ccaaagaagg atgatttccg 60
aaacgaattc gatcacttgt tgatcgaaca ggcaaaccat gctatcgaaa agggagaaca 120
tcaattgctt tacttgcaac accaactcga cgaattgaat gaaaacaaga gcaagggaatt 180
gcaagagaaa atcattcgag aacttgatgt tgtttgcgcc atgatcgaag gagcccaagg 240
agctttggaa cgtgaattga agcgaactga tcttaacatt ttggaacgat tcaactacga 300
agaggctcaa actctcagca agatcttgct taaggatttg aaggaaaccg aacaaaaagt 360
gaaggatatt caaaccat aaaaatttag aattgtacaa ttttacattt ttgatatgat 420
taaattgtcaa taaattgtca ataaataaat tcaattttta actataaaaa aaaaaaaaaa 480
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 509

<210> 65

<211> 461

<212> DNA

<213> *Blomia tropicalis*

<400> 65

ctgctttgcc gcttcggttt tggctcaaga gcacaagcca aagaaggatg atttccgaaa 60
cgaattcgat cacttgttga tcgaacaggc aaaccatgct atcgaaaagg gagaacatca 120
attgctttac ttgcaacacc aactcgacga attgaatgaa aacaagagca aggaattgca 180
agagaaaaatc attcgagaac ttgatgttgt ttgcgccatg atcgaaggag cccaaggagc 240
tttggaacgt gaattgaagc gaactgatct taacattttg gaacgattca actacgaaga 300
ggctcaaact ctcagcaaga tcttgcttaa ggatttgaag gaaaccgaac aaaaagtga 360
ggatattcaa acccaataaa aatttagaat tgtacaattt tacatttttg atatgattaa 420
atgtcaataa atgttcaata aataaaaaaa aaaaaaaaaa a 461

<210> 66

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<211> 434

<212> DNA

<213> *Blomia tropicalis*

<400> 66

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ggagaacatc aattgcttta cttgcaacac caactcgacg aattgaatga aaacaagagc 120
aaggaattgc aagagaaaat cattcgagaa cttgatgttg tttgcgccat gatcgaagga 180
gccaaggag ctttggaacg tgaattgaag cgaactgac ttaacatttt ggaacgattc 240
aactacgaag aggctcaaac tctcagcaag atcttgctta aggatttgaa ggaaaccgaa 300
caaaaagtga aggatattca aacccaataa aaatttagaa ttgtacaatt ttacattttt 360
gatatgatta aatgtcaata aatgttcaat aaataaattc aatttttaac tataaaaaaa 420
aaaaaaaaaa aaaa 434
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<210> 67

<211> 340

<212> DNA

<213> *Blomia tropicalis*

<400> 67

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actagacttg caacaccaac tcgacgaatt gaatgaaaac aagagcaagg aattgcaaga 60
gaaaatcatt cgagaacttg atgttggttg cgccatgac gaaggagccc aaggagcttt 120
ggaacgtgaa ttgaagcgaa ctgatcttaa cattttggaa cgattcaact acgaagaggc 180
tcaaactctc agcaagatct tgcttaagga tttgaaggaa accgaacaaa aagtgaagga 240
tattcaaacc caataaaaat ttagaattgt acaattttac atttttgata tgattaaatg 300
tcaataaatg ttcaataaat aaaaaaaaaa aaaaaaaaaa 340
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<210> 68

<211> 134

<212> PRT

<213> *Blomia tropicalis*

<400> 68

Met Lys Phe Ala Ile Val Leu Ile Ala Cys Phe Ala Ala Ser Val Leu

1

5

10

15

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Ala Gln Glu His Lys Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp
20 25 30

His Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Lys Gly Glu His
35 40 45

Gln Leu Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys
50 55 60

Ser Lys Glu Leu Gln Glu Lys Ile Ile Leu Glu Leu Asp Val Val Cys
65 70 75 80

Ala Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg
85 90 95

Thr Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr
100 105 110

Leu Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val
115 120 125

Lys Asp Ile Gln Thr Gln
130

<210> 69

<211> 134

<212> PRT

<213> Blomia tropicalis

<400> 69

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

Ala Gln Glu His Lys Pro Lys Lys Asp Asp Phe Arg Asn Glu Phe Asp
20 25 30

His Leu Leu Ile Glu Gln Ala Asn His Ala Ile Glu Asn Gly Glu His

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      35              40              45
Gln Leu Leu Tyr Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys
      50              55              60
Ser Lys Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys
      65              70              75              80
Ala Met Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg
              85              90              95
Thr Asp Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr
              100              105              110
Leu Ser Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val
              115              120              125
Lys Asp Ile Gln Thr Gln
      130

<210> 70
<211> 127
<212> PRT
<213> Blomia tropicalis

<400> 70
Asp Lys Thr Val Gly Asn Ser Val Leu Ala Gln Glu His Lys Pro Lys
      1              5              10              15
Lys Asp Asp Phe Arg Asn Glu Phe Asp His Leu Leu Ile Glu Gln Ala
              20              25              30
Asn His Ala Ile Glu Lys Gly Glu His Gln Leu Leu Tyr Leu Gln His
              35              40              45
Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser Lys Glu Leu Gln Glu Lys
              50              55              60
```

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Ile Ile Arg Glu Leu Asp Val Val Cys Ala Met Ile Glu Gly Ala Gln
65 70 75 80

Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr Asp Leu Asn Ile Leu Glu
85 90 95

Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu Ser Lys Ile Leu Leu Lys
100 105 110

Asp Leu Lys Glu Thr Glu Gln Lys Val Lys Asp Ile Gln Thr Gln
115 120 125

<210> 71

<211> 84

<212> PRT

<213> *Blomia tropicalis*

<400> 71

Leu Asp Leu Gln His Gln Leu Asp Glu Leu Asn Glu Asn Lys Ser Lys
1 5 10 15

Glu Leu Gln Glu Lys Ile Ile Arg Glu Leu Asp Val Val Cys Ala Met
20 25 30

Ile Glu Gly Ala Gln Gly Ala Leu Glu Arg Glu Leu Lys Arg Thr Asp
35 40 45

Leu Asn Ile Leu Glu Arg Phe Asn Tyr Glu Glu Ala Gln Thr Leu Ser
50 55 60

Lys Ile Leu Leu Lys Asp Leu Lys Glu Thr Glu Gln Lys Val Lys Asp
65 70 75 80

Ile Gln Thr Gln

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<210> 72

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 72

cccggatcca caatgaagtt cgccatcggtt ctt

33

<210> 73

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 73

gctctagatt attggtttga atatc

25

<210> 74

<211> 507

<212> DNA

<213> *Blomia tropicalis*

<220>

<221> CDS

<222> (9) .. (218)

<400> 74

ggccagag cac ccc gct caa gct ctc gac ttt acc agc tgt gcc cgg atg 50

His Pro Ala Gln Ala Leu Asp Phe Thr Ser Cys Ala Arg Met

1

5

10

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aac gat gga gct ctg gga gcc aag gta gct caa gct gcc tgc atc tcg 98
Asn Asp Gly Ala Leu Gly Ala Lys Val Ala Gln Ala Ala Cys Ile Ser
15 20 25 30

agt tgc aag ttt caa aac tgt ggc acg gga cac tgt gag agg cga ggt 146
Ser Cys Lys Phe Gln Asn Cys Gly Thr Gly His Cys Glu Arg Arg Gly
35 40 45

gga cgt cca acc tgt gtc tgt tct cgc tgt ggc aac ggt ggc ggt gaa 194
Gly Arg Pro Thr Cys Val Cys Ser Arg Cys Gly Asn Gly Gly Gly Glu
50 55 60

tgg ccc aat ctg ccc tcc agg ggt taattatttt gtcatttggt ctgtgaaagt 248
Trp Pro Asn Leu Pro Ser Arg Gly
65 70

gtgacacact atttatatat gctttcaata ggaataacct tcaattttgg tcacactttc 308

ccatcaattt ttgacaattt caaaattaat taaggtttta ttttaaagtt catcaatttt 368

ttgtggcttt attttttttaa aattatccga gatgtttgac agagacaatt gattaattaa 428

aataattttt taaatttaaa aattgtttta taaataaaaa ttttttattt ttggaaaaaa 488

aaaaaaaaaa aaaattcgt 507

<210> 75

<211> 70

<212> PRT

<213> Blomia tropicalis

<400> 75

His Pro Ala Gln Ala Leu Asp Phe Thr Ser Cys Ala Arg Met Asn Asp
1 5 10 15

Gly Ala Leu Gly Ala Lys Val Ala Gln Ala Ala Cys Ile Ser Ser Cys

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	20		25		30										
Lys	Phe	Gln	Asn	Cys	Gly	Thr	Gly	His	Cys	Glu	Arg	Arg	Gly	Gly	Arg
	35		40		45										
Pro	Thr	Cys	Val	Cys	Ser	Arg	Cys	Gly	Asn	Gly	Gly	Gly	Glu	Trp	Pro
	50		55		60										
Asn	Leu	Pro	Ser	Arg	Gly										
	65		70												

<210> 76

<211> 26

<212> DNA

<213> Blomia tropicalis

<400> 76

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26

<210> 77

<211> 26

<212> DNA

<213> Blomia tropicalis

<400> 77

cggaattcctt aacccttgga gggcag

26

<210> 78

<211> 25

<212> DNA

<213> Blomia tropicalis

<400> 78

gaaaagagct ctcgacttta ccagc

25

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<210> 79

<211> 26

<212> DNA

<213> *Blomia tropicalis*

<400> 79

cggaattctt aacccctgga gggcag

26