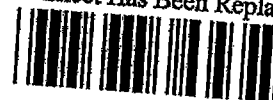


IMMUNOGENIC PROTEINS FROM THE DOMESTIC MITE, *Blomia tropicalis*.

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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
5 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
10 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
15 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
20 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
25 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 <400>36.

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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.

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

A further aspect of the present invention provides a nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence
20 complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or <400>13 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>2 or <400>13.

25 Still another aspect of the present invention provides a nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>28 or <400>30 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in
30 <400>28 or <400>30 wherein said amino acid sequence defines a polypeptide having tropomyosin activity

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Yet another aspect of the present invention provides a nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially
5 as set forth in <400>75 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>75.

Another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>1, or a
10 derivative, homologue or mimetic thereof or capable of hybridizing to <400>1 or its complementary form under low stringency conditions.

A further aspect of the present invention contemplates a nucleic acid molecule encoding *Bt11* or derivative thereof comprising a nucleotide sequence substantially as set forth in
15 <400>1 or <400>2 or <400>13 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>1 or <400>2 or <400>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 <400>2 or <400>13 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13.

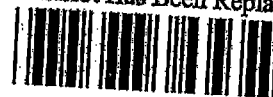
20

Still another aspect of the present invention contemplates a *Bt11*-encoding nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>1 or <400>2 or <400>13.

25 Yet another aspect of the present invention contemplates a genomic nucleic acid molecule or derivative thereof capable of hybridizing to <400>1 or <400>2 or <400>13 or their complementary forms, or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C.

30 Even yet another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>28

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or <400>30, or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>28 or <400>30 or complementary forms thereof under low stringency conditions.

Another aspect of the present invention contemplates a *Bt10*-encoding nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>28 or <400>30 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>28 or <400>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 <400>28 or <400>30 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 and wherein said amino acid sequence defines a polypeptide having tropomyosin activity.

A further aspect of the the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>28 or <400>30.

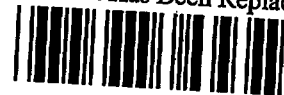
Still another aspect of the present invention contemplates a genomic nucleic acid molecule encoding *Bt10* or derivative thereof capable of hybridizing to <400>28 or <400>30 or complementary forms thereof or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C.

Even yet another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>74, or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>74 or its complementary form under low stringency conditions.

Another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>74 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>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 <400>75 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75.

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A further aspect of the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>74.

5 Still another aspect of the present invention contemplates a genomic nucleic acid molecule or derivative thereof capable of hybridizing to <400>1 or <400>2 or <400>13 or their complemetnary forms or a derivative or homologue or mimetic thereof, under low stringency conditions.

10 Yet another aspect of the present invention is directed to the nucleotide sequence corresponding to *Bt11* is a cDNA or genomic sequence comprising a sequence of nucleotides as set forth in <400>1 or <400>2 or <400>13 or a derivative thereof including a nucleotide sequence having at least about 55% similarity to <400>1 or <400>2 or <400>13.

15

Even yet another aspect of the present invention is directed to the nucleotide sequence corresponding to *Bt10* is a cDNA or genomic sequence comprising a sequence of nucleotides as set forth in <400>28 or <400>30 or a derivative thereof including a nucleotide sequence having at least about 55% similarity to <400>28 or <400>30.

20

A further aspect of the present invention is directed to the nucleotide sequence corresponding to *BtA2* is a cDNA or genomic sequence comprising a sequence of nucleotides as set forth in <400>74 or a derivative thereof thereof including a nucleotide sequence having at least about 55% similarity to <400>74.

25

Still 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
30 equivalent or mimetic thereof;



- (ii) a protein allergen from *B. tropicalis* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- 5 (iii) a protein having an amino acid sequence substantially as set forth in <400>2 or <400>13 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;
- 10 (iv) a protein encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>2 or <400>13 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13 or a derivative, homologue, analogue, chemical equivalent of said protein;
- 15 (v) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in <400>1 or <400>2 or <400>13 or complementary forms thereof or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as
- 20 set forth in <400>2 or <400>13 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13;
- (vi) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a homodimeric
- 25 form; and
- (vii) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a heterodimeric form.
- 30 Yet another aspect of the present invention is directed to Bt10 having tropomyosin activity and an amino acid sequence set forth in <400>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 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>29 or a derivative or homologue or mimetic thereof.

5

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

- 10 (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;
- 15 (iii) a protein having an amino acid sequence substantially as set forth in <400>29 or <400>30 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>29 or <400>30 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;
- 20 (iv) a protein encoded by a nucleotide sequence substantially as set forth in <400>28 or <400>30 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 or a derivative, homologue, analogue, chemical
25 equivalent of said protein;
- (v) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in <400>28 or <400>30 or complementary forms thereof or a derivative or homologue thereof under low stringency conditions and
30 which encodes an amino acid sequence substantially as set forth in <400>28 or <400>30 or a derivative or homologue or mimetic thereof or an amino acid

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sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30;

5 (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.

10 Still another aspect of the present invention is directed to BtA2 having an amino acid sequence set forth in <400>75 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 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>75 or a derivative or
15 homologue or mimetic thereof.

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

20 (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;

25

(iii) a protein having an amino acid sequence substantially as set forth in <400>75 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;

30

(iv) a protein encoded by a nucleotide sequence substantially as set forth in <400>74 or

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a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>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 <400>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 <400>75 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75;

10

(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 a defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a heterodimer form.

Even yet another aspect of the present invention provides a method of preventing, reducing or otherwise ameliorating a Bt hypersensitivity condition in a subject, said method comprising 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.

20

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, down-regulate or otherwise modulate said immune response.

25
30

A further aspect of the present invention extends to the use of Bt in the manufacture of a

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medicament for modulating an immune response.

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

5

Yet 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.

10 Even yet another aspect of the present invention provides a composition comprising 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 Another aspect of the present invention is directed to antibodies to Bt and their derivatives, homologues, analogues, mimetics and chemical equivalents thereof.

A further 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 comprising
20 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.

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

25

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.

30 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).

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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

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TABLE 2

SEQUENCE IDENTIFIER	DESCRIPTION
<400>1	Nucleotide sequence of clone encoding Bt11
<400>2	Nucleotide and corresponding amino acid sequences of clone encoding Bt11
<400>3 to <400>12	Amino acid sequences encoded by <400>2
<400>13	Nucleotide sequence and corresponding amino acid sequence of <i>Bt11</i> gene
<400>14	Amino acid sequence of Bt11
<400>15	Amino acid sequence of <i>Dermatophagoides farinae</i> paramyosin
<400>16	Amino acid sequence of <i>Drosophila melanogaster</i> paramyosin
<400>17	Amino acid sequence of <i>Onchocerca volvulus</i> paramyosin
<400>18	Amino acid sequence of <i>Caenorhabditis elegans</i> paramyosin
<400>19	T3 primer
<400>20	T7 primer
<400>21	Gene-specific primer 1 (GSP1)
<400>22	Gene-specific primer 2 (GSP2)
<400>23	Nested universal primer (NUP)
<400>24	T7 primer
<400>25	Bt11 CS2f primer
<400>26	Bt11 R2 primer
<400>27	Bt11 polymorphic variant
<400>28	Nucleotide sequence of clone encoding Bt10
<400>29	Amino acid sequence encoded by <400>28
<400>30	Nucleotide sequence of coding region of <400>28
<400>31	Amino acid sequence encoded by <400>30
<400>32	Amino acid sequence of Group 10 allergen from <i>Dermatophagoides farinae</i> (Df10)
<400>33	Amino acid sequence of Group 10 allergen from <i>Lepidoglyphus destructor</i> (Lp10)

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SEQUENCE IDENTIFIER	DESCRIPTION
<400>34	Amino acid sequence of Group10 allergen from <i>Dermatophagoides pteronyssinus</i> (Dp10)
<400>35	Amino acid sequence of Group 10 allergen from <i>Dermatophagoides pteronyssinus</i> (Dp10)
<400>36	Bt5 nucleotide sequence (ref 17)
<400>37	Bt5 amino acid sequence (ref 17)
<400>38	Bt5 polymorphic genomic sequence no. 1
<400>39	Bt5 polymorphic genomic sequence no. 2
<400>40	Bt5 polymorphic genomic sequence no. 3
<400>41	Bt5 polymorphic genomic sequence no. 4
<400>42	Bt5 polymorphic genomic sequence no. 5
<400>43	Bt5 polymorphic genomic sequence no. 6
<400>44	Bt5 polymorphic genomic sequence no. 7
<400>45	Bt5 polymorphic genomic sequence no. 8
<400>46	Bt5 polymorphic genomic sequence no. 9
<400>47	Bt5 polymorphic genomic sequence no. 10
<400>48	Bt5 polymorphic genomic sequence no. 11
<400>49	Bt5 polymorphic amino acid sequence nos. 1-3 and 7-10
<400>50	Bt5 polymorphic amino acid sequence no. 4
<400>51	Bt5 polymorphic amino acid sequence no. 5
<400>52	Bt5 polymorphic amino acid sequence no. 6
<400>53	Bt5 polymorphic amino acid sequence no. 11
<400>54	Bt5 leader sequence (ref 17)
<400>55	Bt5 cDNA variant no. 1(13)
<400>56	Bt5 cDNA variant no. 2(7)
<400>57	Bt5 cDNA variant no. 3(2)
<400>58	Bt5 cDNA variant no. 4(15)
<400>59	Bt5 cDNA variant no. 5(1)
<400>60	Bt5 cDNA variant no. 6(11)
<400>61	Bt5 cDNA variant no. 7(4)



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SEQUENCE IDENTIFIER	DESCRIPTION
<400>62	Bt5 cDNA variant no. 8(12)
<400>63	Bt5 cDNA variant no. 9(24)
<400>64	Bt5 cDNA variant no. 10(9)
<400>65	Bt5 cDNA variant no. 11(3)
<400>66	Bt5 cDNA variant no. 12(5)
<400>67	Bt5 cDNA variant no. 13(10)
<400>68	Bt5 polymorphic amino acid variant no. 7
<400>69	Bt5 polymorphic amino acid variant no. 24
<400>70	Bt5 polymorphic amino acid variant no. 13
<400>71	Bt5 polymorphic amino acid variant no. 10
<400>72	DNA primer
<400>73	DNA primer
<400>74	Nucleotide sequence of clone encoding BtA2
<400>75	Amino acid sequence encoded by <400>74
<400>76	Primer sequence
<400>77	Primer sequence
<400>78	Primer sequence
<400>79	Primer sequence

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TABLE 3

Variant	Position	Residue	Hyrdophobic *	Charge	Chemical
<u>Genomic</u>					
<400>36,38-41 & 43-47	4	Lys	Phi	positive	basic
<400>42	4	Glu	Phi	negative	acidic
<400>36, 38-42 & 44-47	12	Asn	Phi	neutral	amide
<400>43	12	Asp	Phi	negative	acidic
<400>36, 38-40 & 42-10	17	Leu	Pho	neutral	aliphatic
<400>41	17	Trp	Pho	neutral	aromatic
<400>36, 38-41 & 43-47	33	Leu	Pho	neutral	aliphatic
<400>43	33	Ile	Pho	neutral	aliphatic
<400>36, 38-42 & 44-47	34	Leu	Pho	neutral	aliphatic
<400>43	34	His	Phi	positive	basic
<400>36, 38-42 & 44-47	117	Gln	Phi	neutral	aliphatic
<400>43	117	Lys	Phi	positive	basic
<400>36, 38-47	2	Glu	Phi	negative	acidic
<400>48	2	Gln	Phi	neutral	aliphatic
<u>cDNA</u>					
<400>36, 56 & 67	28	Lys	Phi	positive	basic
<400>63	28	Asn	Phi	neutral	amide
<400>36, 56 & 63	35	Tyr	Pho	neutral	aromatic
<400>67	35	Asp	Phi	negative	acidic
<400>36, 63 & 67	57	Arg	Phi	positive	basic
<400>56	57	Leu	Pho	neutral	aliphatic

* Phi: hydrophilic and Pho: hydrophobic

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BRIEF DESCRIPTION OF THE FIGURES

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

5

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

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.

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*.

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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 μ g per
10 dot is loaded for recombinant Bt11 and recombinant GST; 5 μ g 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.

15

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.

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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 (<400>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 (<400>74 and <400>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 kD. From 1 litre culture of *E. coli*, 7-10 mg of GST-clone BtA2 fusion protein could be
10 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 chromatography and two lanes with different concentrations of elute (GST-BtA2 fusion
15 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 medium of yeast culture.

20

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 after thrombin digestion of the GST-BtA2 fusion protein; (2) 2 µg of GST-BtA2 fusion
25 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 variants. The peptides were designed to be 16-amino acid residues in length, overlapping
30 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.



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These peptide variants contain naturally occurring mutations of Bt5 proteins.

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 at 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 sequence.

10

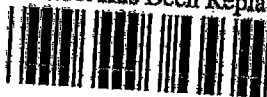
Figure 22 shows the comparison of the deduced amino acid sequences for the Bt5 cDNA variants. Number on the right indicates the position of the amino acid residue.

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.

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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 protein allergen from a 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.

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 *B. tropicalis* or a derivative, homologue or mimetic of said protein allergen.

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

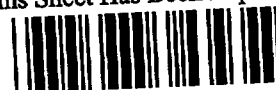
- 25 -

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 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 <400>38 to <400>53
5 (genomic sequences), <400>59 to <400>67 (cDNA sequences) and <400>68 to <400>71 (amino acid sequences).

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
10 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 immune response is directed. It may also, or alternatively, comprise one or more peptide
15 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 immune response in all individuals who are exposed to it. It should also be understood that
20 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 tolerance.

25 In one preferred embodiment, the present invention provides a nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>2 or <400>13 or a derivative, homologue or mimetic thereof or having
30 at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>2 or <400>13. This protein is referred to herein as "Bt11". The corresponding gene is "Bt11".

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In another preferred embodiment, the present invention provides a nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially
5 as set forth in <400>28 or <400>30 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>28 or <400>30 wherein said amino acid sequence defines a polypeptide having tropomyosin activity. This protein is referred to herein as "Bt10". The corresponding gene is "Bt10".

10

In another preferred embodiment, the present invention provides a nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially
15 as set forth in <400>75 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>75. 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".

20

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
25 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.

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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 "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.

10

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 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 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 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 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.

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Another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>1, or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>1 or its
5 complementary form under low stringency conditions. This nucleotide sequence corresponds to "*Bt11*".

Preferably, the present invention contemplates a nucleic acid molecule encoding *Bt11* or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>1 or
10 <400>2 or <400>13 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>1 or <400>2 or <400>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 <400>2 or <400>13 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13.

15

An example of a derivative is a polymorphic variant defined of *Bt11* is by the nucleotide sequence set forth in <400>27 and with a corresponding amino acid sequence set forth in <400>27.

20 More particularly, the present invention contemplates a *Bt11*-encoding nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>1 or <400>2 or <400>13.

Another aspect of the present invention contemplates a genomic nucleic acid molecule or
25 derivative thereof capable of hybridizing to <400>1 or <400>2 or <400>13 or their complementary forms, or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C.

Reference herein to *Bt11* and *Bt11* should be understood as a reference to all forms of *Bt11*
30 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*

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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. 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 <400>27 and its
5 corresponding amino acid sequence <400>27.

Another aspect of the present invention contemplates a nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>28 or <400>30, or a derivative, homologue or mimetic thereof or capable of hybridizing to
10 <400>28 or <400>30 or complementary forms thereof under low stringency conditions. This nucleic acid molecule is referred to herein as "*Bt10*".

Preferably, the present invention contemplates a *Bt10*-encoding nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>28
15 or <400>30 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>28 or <400>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 <400>28 or <400>30 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 and wherein said amino acid sequence
20 defines a polypeptide having tropomyosin activity.

More particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>28 or <400>30. This nucleic acid molecule is referred to herein as "*Bt10*". Reference herein to "*Bt10*" includes both a
25 cDNA molecule and a genomic gene as well as any polymorphic variants thereof.

Another aspect of the present invention contemplates a genomic nucleic acid molecule encoding Bt10 or derivative thereof capable of hybridizing to <400>28 or <400>30 or complementary forms thereof or a derivative or homologue or mimetic thereof, under low
30 stringency conditions of 42°C. The genomic nucleic acid molecule preferably encodes a tropomyosin.

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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 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 <400>74, or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>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 <400>74 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>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 <400>75 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75.

Even more particularly, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in <400>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.

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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
5 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*.

10 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
15 acid sequence or its encoding nucleotide sequence as set forth above (see also Table 3). 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.

20 Another aspect of the present invention contemplates a genomic nucleic acid molecule or derivative thereof capable of hybridizing to <400>1 or <400>2 or <400>13 or their complementary forms or a derivative or homologue or mimetic thereof, under low stringency conditions.

25 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
30 derivatives, homologs, analogues, chemical equivalents and mimetics thereof.

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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 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.

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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.

5

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.

10

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; 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 .

20

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.

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

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-

30





chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

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

- Examples of incorporating unnatural amino acids and derivatives during protein synthesis
- 15 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
- 20 Patent Publication No. WO 97/15663].

- 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
- 25 reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety.

- 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
- 30 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,

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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
5 may also be considered, in a preferred embodiment, to be biologically pure.

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
10 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.

15 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 <400>1 or <400>2 or <400>13 or a derivative thereof including a nucleotide sequence having at least about 55% similarity to <400>1 or <400>2 or <400>13.

20 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 <400>28 or <400>30 or a derivative thereof including a nucleotide sequence having at least about 55% similarity to <400>28 or <400>30.

25 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 <400>74 or a derivative thereof thereof including a nucleotide sequence having at least about 55% similarity to <400>74.

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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 <400>1 or <400>2 or <400>13 or <400>28 or <400>30 or <400> 38 to <400> 48 or <400>55 to <400>67 or <400>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 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 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.

One expression product is Bt11 having an amino acid sequence set forth in <400>2 or <400>13 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 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>2 or <400>13 or a derivative or homologue or mimetic thereof.

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;

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- (ii) a protein allergen from *B. tropicalis* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;
- 5 (iii) a protein having an amino acid sequence substantially as set forth in <400>2 or <400>13 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;
- 10 (iv) a protein encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>2 or <400>13 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13 or a derivative, homologue, analogue, chemical equivalent of said protein;
- 15 (v) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in <400>1 or <400>2 or <400>13 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 <400>2 or <400>13 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13;
- 20 (vi) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a homodimeric form; and
- 25 (vii) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a heterodimeric form.
- 30

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Yet another expression product is Bt10 having tropomyosin activity and an amino acid sequence set forth in <400>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 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>29 or a derivative or homologue or mimetic thereof.

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

10

- (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;
- (iii) a protein having an amino acid sequence substantially as set forth in <400>29 or <400>30 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>29 or <400>30 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 <400>28 or <400>30 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 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 <400>28 or <400>30 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 <400>28 or <400>30 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30;

5

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

10

(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 <400>75 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 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>75 or a derivative or homologue or mimetic thereof.

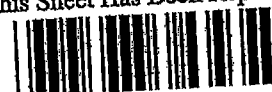
Accordingly, another aspect of the present invention is directed to an isolated protein selected from the list consisting of:-

(viii) a protein allergen from a mite or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;

25 (ix) a protein allergen from *B. tropicalis* or a derivative, homologue, analogue, chemical equivalent or mimetic thereof;

(x) a protein having an amino acid sequence substantially as set forth in <400>75 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;

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- (xi) a protein encoded by a nucleotide sequence substantially as set forth in <400>74 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75 or a derivative, homologue, analogue or chemical equivalent of said protein;
- (xii) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in <400>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 <400>75 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>75;
- (xiii) a protein as defined in paragraphs (i) or (ii) or (iii) or (iv) or (v) in a homodimeric form; and
- (xiv) 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.

- 25 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 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,
- 30 another molecule in an amount capable of inducing tolerance to an allergen.

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The ability to produce recombinant Bt permits the large scale production of Bt for 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 extraneous proteinaceous sequences. Such processing includes digestion with proteases, peptidases and amidases or a range of chemical, electrochemical, sonic or mechanical disruption techniques.

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

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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.

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.

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 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.

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Accordingly, the present invention provides a method of preventing, reducing or otherwise ameliorating a Bt hypersensitivity condition in a subject said method comprising 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
5 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
10 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
15 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).

20 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
25 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
30 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

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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.

Accordingly, another aspect of the present invention relates to a method of modulating, in
5 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, down-regulate or otherwise modulate said immune response.

10 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 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,
15 where it is sought to induce an immune response, doses of Bt which do not induce tolerance may be administered.

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

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

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

25

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 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
30 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*



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the same or different routes or sequential administration by the same or different routes. By "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.

5

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

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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

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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

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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

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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.

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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 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.

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, 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 is such that a suitable dosage will be obtained. Preferred compositions or 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 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



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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.

- 5 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 be incorporated into sustained-release preparations and formulations.

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

- 15 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 contemplated. Supplementary active ingredients can also be incorporated into the compositions.

- 20 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 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
25 and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved and (b) the limitations inherent in the art of compounding such an active material.

- 30 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

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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.

Preferably, the nucleotide sequence encodes the amino acid sequence of Bt11 substantially as set forth in <400>2 or <400>13 or a derivative, homologue or mimetic thereof or having
15 at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>2 or <400>13.

Even more preferably, the nucleotide sequence comprises the *Bt11* nucleotide sequence of <400>1 or <400>2 or <400>13, or a derivative, homologue or mimetic thereof, or capable
20 of hybridizing to <400>1 or <400>2 or <400>13 under low stringency conditions.

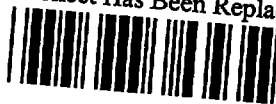
Preferably, the nucleotide sequence encodes the amino acid sequence of Bt10 substantially as set forth in <400>28 or <400>30 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in
25 <400>28 or <400>30 and which corresponds to a polypeptide having tropomyosin activity.

Even more preferably, the nucleotide sequence comprises the *Bt10* nucleotide sequence of <400>28 or <400>30 or a derivative, homologue or mimetic thereof, or capable of hybridizing to <400>28 or <400>30 under low stringency conditions.

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Preferably, the nucleotide sequence encodes the amino acid sequence of BtA2 substantially





as set forth in <400>75 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>75.

Even more preferably, the nucleotide sequence comprises the *BtA2* nucleotide sequence of
5 <400>74, or a derivative, homologue or mimetic thereof, or capable of hybridizing to <400>74 under low stringency conditions.

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

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Still another aspect of the present invention contemplates a method for the prophylactic 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.

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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 human such as mite allergens, glutathione S-transferase, pollen, animal dander, house dust and peanut and the like.

25

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

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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.

5

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.

10

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 the intramuscular injection of a plasmid DNA encoding a house dust or storage mite allergen (28).

15

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

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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 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.

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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
5 herein to include fragments and hybrids of antibodies.

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.

10

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-
15 immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of Bt.

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
20 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 utilizable in virtually any type of immunoassay, they are generally less favoured because
25 of the potential heterogeneity of the product.

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 preparation of hybridoma cell lines for monoclonal antibody production derived by fusing
30 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.

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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.

- 5 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 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.

10

- Detection of the presence of Bt (for example, in a dust sample) or antibody to Bt may be 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 to a target, such as Bt.
- 15

- 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 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 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
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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

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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.

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



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- 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
- 5 the presence of the hapten of interest. Immunofluorescence and EIA techniques are both 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.
- 10 Further features of the present invention are more fully described in the following non-limiting Examples.

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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
5 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
10 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
15 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-
20 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
25 (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
30 at the bottom of the sieves were used for further subculturing.



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"Examples 2 to 13 relate to Bt11"

EXAMPLE 2

Screening of Bt cDNA Library

5

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 μ Joules 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 (Boehringer 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 μ l 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') (<400>19)
or T7 primer (3'CGGGATATCACTC-AGCATAATG5') (<400>20) and 8 µl of Big Dye
25 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).



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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.

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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 µl 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' (<400>21), and Gene-Specific Primer 2 (GSP2), 5'GGTCGCTCAGAAGACGGTAC3' (<400>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



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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' (<400>23), and the T7 primer, 3'CGGGATATCACTCAGC-ATAATG5' (<400>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 [Trademark]
25 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).

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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 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 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 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 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 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 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

5 *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

10 expression vector plasmid. Primer Bt11CS2f,
 5'CGGGATCCATGGCGGCTCGATCAGCA3' (<400>25), was used to create a BamH1
 restriction site on the 5'end while Primer Bt11R2,
 3'CGCTCGAGTTAAAAGTTCGAGCTCTCTTG5' (<40026>), was used to create an
 Xho1 restriction site at the 3' end. Pfu polymerase was used in the amplification process.

15 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

20 extraction. Purified Bt11 insert was ligated overnight at 16°C into PGEX-4T-1 vector
 (Pharmacia Biotech) previously digested with *Bam*H1 and *Xho*1. 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

25 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.

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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 Whatmanni 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



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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'-

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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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- 20

25

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.

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**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.

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

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

EXAMPLE 15

5

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 10 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, 15 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.

20

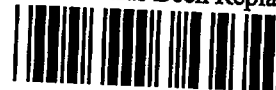
EXAMPLE 16

DNA sequencing of Bt10 cDNA clone

The *Bt10*-containing phagemid was excised *in vivo* using fl helper phage as described in 25 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).

15

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.

25

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



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 ExtraAvidin 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.

10

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.

20

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

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to as "Bt10". The nucleotide and corresponding amino acid sequence of *Bt10* are defined in <400>28 and <400>29, respectively.

EXAMPLE 23

5

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*
10 *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.

15

EXAMPLE 24

IgE plaque immunoassay

Nine out of 13 mite-allergic individuals' sera used in this assay showed a positive result,
20 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

25

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
30 reported by Aki *et al.* (29).

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

The ability of IgE-binding of recombinant fusion Bt10 was determined by Western
5 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

15

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.

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**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.

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

25 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).

15

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

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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

5 *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* (Invitrogen) following the protocols of the manufacturers.

10

EXAMPLE 36

Expression of recombinant BtA2 clone in E. coli

The BtA2 clone was amplified by PCR using the primers 5'-
15 CGGGATCCGCTCTCGACTTTACCAGC-3' (*Bam*H1 site in italics) [<400> 76] and 5'-
CGGAATTCTTAACCCCTGGAGGGCAG-3' (*Eco*R1 site in italics) [<400> 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
20 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,
25 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
30 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.

EXAMPLE 37

5 *Expression of recombinant BtA2 clone in the yeast Pichia Pastoris*

The BtA2 clone was amplified by PCR using the primers 5'-GAAAAGAGCTCTCGACTTTACCAGC-3' [$<400>$ 78] and 5'-CGGAATTCTTAACCCCTGGAGGGCAG-3' (*EcoR*I site in italics) [$<400>$ 79], then the
10 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 with *EcoR*I. After ligation, it was transformed into *P. pastoris*. GS115 (Invitrogen), screening
15 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 for their ability to grow on histidine-deficient medium. Transformants of GS1154 with Bgl
20 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 readily distinguished. Several transformants were then grown overnight in 1 ml of BMGY
25 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 BMMY medium (0.5% v/v methanol replaces the glycerol of BMGY medium) with its
30 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



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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 grow in large volume for mass extraction of recombinant A2 clone protein.

5

EXAMPLE 38

SDS-PAGE

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 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).

15

EXAMPLE 39

Sequence analysis

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

25

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 recombination protein with a stop codon (TAA) at nucleotide residues 219-221. The deduced peptide has a cycteine rich between nucleotide residues 70 to 200 as detected in the databases of the National Center for Biotechnology

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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. The sample was cooled on ice for 10 minutes. The PCR reaction was carried out in 1 X
15 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' <400>35 (primer) and 5'GCTCTAGATTATTGGTTTGAATATC3' <400>36 (primer), respectively. The reaction was heated to 95°C for 5 minutes, annealed at 57°C for 2 minutes, and
20 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 into the pCR2.1 vector using the TA cloning kit (Invitrogen). Sequence of the Bt5 genomic
25 gene was determined by ABI PRISM™ DNA sequencing kit.

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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.

15

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.

25

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).

20

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

30



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

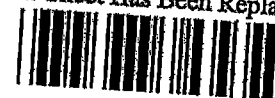
EXAMPLE 47

5

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
10 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
15 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
20 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
25 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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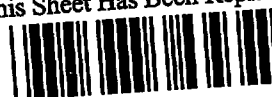
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**CLAIMS**

1. An isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding or complementary to a sequence encoding protein allergen from a mite or a derivative, homologue or mimetic of said protein allergen.
2. An isolated nucleic acid molecule according to Claim 1 wherein the mite is a dust or storage mite or a storage mite.
3. An isolated nucleic acid molecule according to Claim 1 wherein the dust or storage mite is *Blomia tropicalis*.
4. A nucleic acid molecule according to Claim 2 comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400> 2 or <400>13 or a derivative, homologue or mimetic thereof or having at least about 45% or greater similarity to at least 10 contiguous amino acids in <400> 2 or <400>13.
5. A nucleic acid molecule according to Claim 2 comprising a nucleotide sequence substantially as set forth in <400>1 or <400>2 or <400>13, or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>1 or <400>2 or <400>13 under low stringency conditions.
6. A nucleic acid molecule according to Claim 2 comprising a nucleotide sequence encoding an amino acid sequence set forth in <400>27.
7. A nucleic acid molecule according to Claim 6 comprising a nucleotide sequence substantially as set forth in <400>27.
8. A nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>1 or <400>2 or <400>13 or a derivative,

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homologue or mimetic thereof or capable of hybridizing to <400>1 or <400>2 or <400>13 under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>2 or <400>13 or a sequence having at least about 45% similarity to at least 10 contiguous amino acids in <400>2 or <400>13.

9. A nucleic acid molecule according to Claim 6 comprising a sequence of nucleotides substantially as set forth in <400>1 or <400>2 or <400>13.

10. A nucleic acid molecule according to Claim 6 comprising a nucleotide sequence substantially as set forth in <400>27.

11. A genomic nucleic acid molecule or derivative thereof capable of hybridizing to <400>1 or <400>2 or <400>13, or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C.

12. A genomic nucleic acid molecule or derivative thereof capable of hybridizing to <400>27 or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C.

13. A cDNA sequence comprising a sequence of nucleotides as set forth in <400>1 or <400>2 or <400>13 or a derivative thereof including a nucleotide sequence having similarity to <400>1 or <400>2 or <400>13.

14. A cDNA sequence comprising a sequence of nucleotides as set forth in <400>27 or a derivative thereof including a nucleotide sequence having similarity to <400>27.

15. A nucleic acid molecule according to Claim 2 comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>28 or <400>30 or a





derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>28 or <400>30.

16. A nucleic acid molecule according to Claim 2 comprising a nucleotide sequence substantially as set forth in <400>28 or <400>30, or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>28 or <400>30 under low stringency conditions.

17. A nucleic acid molecule according to Claim 2 wherein the nucleic acid molecule is a polymorphic variant of the nucleotide sequence set forth in <400>28 or <400>30.

18. A nucleic acid molecule or derivative thereof comprising a nucleotide sequence substantially as set forth in <400>28 or <400>30 or a derivative, homologue or mimetic thereof or capable of hybridizing to <400>28 or <400>30 under low stringency conditions and which encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>28 or <400>30 or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 and wherein said amino acid sequence defines a polypeptide having tropomyosin activity.

19. A nucleic acid molecule according to Claim 18 comprising a sequence of nucleotides substantially as set forth in <400>28 or <400>30.

20. A genomic nucleic acid molecule or derivative thereof capable of hybridizing to <400>28 or <400>30, or a derivative or homologue or mimetic thereof, under low stringency conditions of 42°C and wherein said nucleic acid molecule encodes a polypeptide having tropomyosin activity.

21. A cDNA sequence comprising a sequence of nucleotides as set forth in <400>28 or <400>30 or a derivative thereof including a nucleotide sequence having





similarity to <400>28 or <400>30 and wherein said cDNA sequence encodes a polypeptide having tropomyosin activity.

22. An isolated nucleic acid molecule or derivative thereof comprising a nucleotide sequence encoding, or a nucleotide sequence complementary to a nucleotide sequence encoding, an amino acid sequence substantially as set forth in <400>75 or a derivative, homologue or mimetic thereof or having at least about 55% or greater similarity to at least 10 contiguous amino acids in <400>75.

23. An isolated nucleic acid molecule comprising a nucleotide sequence substantially as set forth in <400>74, or a derivative, homologue or mimetic thereof, or capable of hybridizing to <400>74 under low stringency conditions.

24. An isolated nucleic acid molecule according to Claim 23 which further encodes an amino acid sequence corresponding to an amino acid sequence set forth in <400>75 or a sequence having at least 55% similarity to at least 10 contiguous amino acids in <400>75.

25. An isolated nucleic acid molecule according to Claim 23 or 24 substantially as set forth in <400>74.

26. A protein having an amino acid sequence set forth in <400>2 or <400>13 or is a derivative, homologue, analogue, chemical equivalent or mimetic thereof having at least about 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>2 or <400>13 or a derivative or homologue or mimetic thereof.

27. A protein having an amino acid sequence set forth in <400>27 or is a derivative, homologue, analogue, chemical equivalent or mimetic thereof having at least about 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>27 or a derivative or homologue or mimetic thereof.





28. 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;
- (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 <400>2 or <400>13 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13 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 <400>1 or <400>2 or <400>13 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>2 or <400>13 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 <400>1 or <400>2 or <400>13 or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set further in <400>1 or <400>2 or <400>13 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 <400>1 or <400>2 or <400>13;

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

29.

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;
- (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 <400>27 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>27 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 <400>27 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 <400>27 or a derivative, homologue, analogue, chemical equivalent of said protein;
- (iv) a protein encoded by a nucleic acid molecule capable of hybridizing to the nucleotide sequence as set forth in <400>27 or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set further in <400>27 or a derivative or homologue or mimetic thereof or an





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amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>27;

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

30. A protein having an amino acid sequence set forth in <400>28 or <400>30 or is a derivative, homologue, analogue, chemical equivalent or mimetic thereof having at least about 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>28 or <400>30 or a derivative or homologue or mimetic thereof and wherein said amino acid sequence defines a polypeptide having tropomyosin activity.

31. 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;
- (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 <400>28 or <400>30 or a derivative, homologue or mimetic thereof or a sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein;

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- (iv) a protein encoded by a nucleotide sequence substantially as set forth in <400>28 or <400>30 or a derivative or homologue thereof or a sequence encoding an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30 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 <400>28 or <400>30 or a derivative or homologue thereof under low stringency conditions and which encodes an amino acid sequence substantially as set forth in <400>28 or <400>30 or a derivative or homologue or mimetic thereof or an amino acid sequence having at least about 55% similarity to at least 10 contiguous amino acids in <400>28 or <400>30;
- (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,

and wherein said protein has tropomyosin activity.

32. An isolated protein comprising an amino acid sequence as set forth in <400>75 or a derivative, homologue, analogue, chemical equivalent or mimetic thereof or having at least about 55% similarity to at least 10 contiguous amino acids in the amino acid sequence as set forth in <400>75 or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.

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33. An isolated protein according to Claim 32 encoded by a nucleotide sequence substantially as set forth in <400>74 or a derivative or homologue thereof or capable of hybridizing to <400>74 under low stringency conditions or a derivative, homologue, analogue, chemical equivalent or mimetic of said protein.
34. An isolated protein according to Claim 32 or 33 substantially as set forth in <400>75.
35. An isolated protein according to Claim 32 or 33 in homodimeric form.
36. An isolated protein according to Claim 32 or 33 in heterodimeric form.
37. 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.
38. 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.
39. 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.
40. A method according to Claim 39 wherein the allergic condition is allergic asthma, atopic dermatitis and/or rhinitis.

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41. A method according to Claim 39 or 40 wherein the nucleic acid molecule is in the form of an eukaryotic expression vector.
42. The method of Claim 39 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.
43. The method of Claim 39 wherein the individual is a human.
44. The method of Claim 39 wherein the vector is a plasmid vector.
45. A method according to Claim 39 wherein the administration is *via* intramuscular injection.
46. Use of a *B. tropicalis* allergen in the manufacture of a medicament for modulating an immune response.
47. An agent useful for modulating an immune response, said agent comprising a *B. tropicalis* allergen.
48. A composition for use in modulating an immune response comprising a *B. tropicalis* allergen and one or more pharmaceutically acceptable carriers and/or diluents.
49. Antibodies to a *B. tropicalis* allergen and its derivatives, homologues, analogues, mimetics and chemical equivalents thereof.
50. 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,

