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(54) Title: LUTZOMYIA LONGIPALPIS POLYPEPTIDES AND METHODS OF USE

(57) Abstract: Substantially purified salivary lu. longipalpis polypeptides, and polynucleotides encoding these polypeptides are disclosed. Vectors and host cells including the lu. longipalpis polynucleotides are also disclosed. In one embodiment, a method is disclosed for inducing an immune response to sand fly saliva. In other embodiments, methods for treating, diagnosing, or preventing leishmaniasis are disclosed.

**LUTZOMYIA LONGIPALPIS POLYPEPTIDES AND METHODS OF USE****PRIORITY CLAIM**

5 This application claims the benefit of U.S. Provisional Application No. 60/422,303, filed October 29, 2002, which is incorporated herein by reference.

**FIELD**

10 The disclosure relates to proteins substantially purified from *Lutzomyia longipalpis* (*Lu. longipalpis*) sand fly salivary glands, or recombinant vectors expressing these proteins, and to an immune response produced to these proteins. This disclosure also relates to the production of an immune response that affects survival of *Leishmania*.

15 **BACKGROUND**

Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania* and affect many millions of people worldwide. In humans, infection with the parasite manifests either as a cutaneous disease caused mainly by *L. major*, *L. tropica*, and *L. mexicana*; as a mucocutaneous disease caused mainly by *L. brasiliensis*; or as a visceral disease caused mainly by *L. donovani* and *L. 20 chagasi*. In canids, *Leishmania* infections manifest as a visceral disease that can result in high death rates.

25 All leishmanial diseases are transmitted to their vertebrate hosts by phlebotomine sand flies, which acquire the pathogen by feeding on infected hosts and transmit them by regurgitating the parasite at the site of a subsequent blood meal (Killick-Kendrick, *Biology of Leishmania in phlebotomine sand flies*. In *Biology of the Kinetoplastida*. W. Lumsden and D. Evans, editors. Academic Press, New York. 395, 1979).

30 While obtaining a blood meal, sand flies salivate into the host's skin. This saliva contains anticoagulant, antiplatelet, and vasodilatory compounds that increase the hemorrhagic pool where sand flies feed (Ribeiro *et al.*, *Comp. Biochem. Physiol.* 4:683, 1986; Charlab *et al.*, *Proc. Natl. Acad. Sci. USA*. 26:15155, 1999). Some of these components are additionally immunomodulatory. For example, the New World sand fly *Lutzomyia longipalpis* contains the 6.5 kDa peptide, maxadilan, which is the most potent vasodilator known (Lerner *et al.*, *J. Biol. Chem.* 17:11234, 1991). Maxadilan additionally has immunosuppressive activities of its own (Qureshi *et al.*, *Am. J. Trop. Med. Hyg.* 6:665, 1996), as do many persistent vasodilators such as prostaglandin E<sub>2</sub> (Makoul *et al.*, *J. Immunol.* 134:2645, 1985; Santoli and Zurier, *J. Immunol.* 143:1303, 1989; Stockman and Mumford, *Exp. Hematol.* 2:65, 1974) and calcitonin gene-related peptide (Nong *et al.*, *J. Immunol.* 1:45, 1989). Old World sand flies do not have maxadilan but instead use AMP and adenosine as vasodilators (Ribeiro *et al.*, *J. Exp. Biol.* 11:1551, 1999). Adenosine is also an immunomodulatory component, promoting the production of IL-10 and suppressing TNF- $\alpha$  and IL-12 in mice (Hasko *et al.*, *J. Immunol.* 10:4634, 1996; Webster, *Asian Pac. J. Allergy Immunol.* 2:311, 1984; Hasko *et al.*, *40*

*FASEB J.* 14:2065, 2000). Despite what is known about the role of sand fly saliva and disease transmission, much remains unknown, and an effective vaccine does not exist. Thus, there is a need for agents that can be used to induce an immune response to the organisms that cause leishmaniasis.

5

## SUMMARY

The present disclosure relates to salivary proteins from sand fly vectors of *Lutzomyia longipalpis* (*Lu. longipalpis*) and the nucleic acids that encode these proteins. Methods of producing an immune response in a subject are also disclosed.

Substantially purified salivary *Lu. longipalpis* polypeptides are disclosed herein. Also 10 disclosed are polynucleotides encoding the *Lu. longipalpis* polypeptides.

Methods are disclosed for inducing an immune response using a therapeutically effective amount of a substantially purified salivary *Lu. longipalpis* polypeptide as disclosed herein, or the polynucleotide encoding a *Lu. longipalpis* polypeptides disclosed herein.

In another embodiment methods are disclosed herein for inhibiting the symptoms of a 15 *Leishmania* infection or for preventing a *Leishmania* infection in a subject. The methods include administering to the subject a therapeutically effective amount of a *Lu. longipalpis* polypeptide, or a polynucleotide encoding a *Lu. longipalpis* polypeptide. In two non-limiting examples, more than one *Lu. longipalpis* polypeptide can be administered, or at least one *Lu. longipalpis* polypeptide in conjunction with a *P. ariasi* or *P. perniciosus* polypeptide.

20 Also disclosed herein are methods of diagnosing *Leishmania* infection in a subject. The methods include contacting a solid substrate comprising at least three, six, or ten *Lu. longipalpis* polypeptides, or an immunogenic fragment thereof, contacting the solid substrate with a sample obtained from the subject and detecting binding of a component of the sample to at least one polypeptide on the solid substrate. Detection of binding of the component to the substrate indicates 25 that the subject is infected with *Leishmania*.

Pharmaceutical compositions are disclosed including a pharmaceutically acceptable carrier and a *Lu. longipalpis* polypeptide.

The foregoing and other features and advantages will become more apparent from the 30 following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

## BRIEF DESCRIPTION OF THE FIGURES

**FIG. 1** is a set of bar graphs showing the levels of antibodies against *Lutzomyia longipalpis* (*Lu. longipalpis*) saliva in sera of individuals. Human sera were obtained at time 0 (negative anti-35 *Leishmania* serology ( $S^-$ ) or negative DTH ( $DTH^-$ )) and 6 months later (positive anti-*Leishmania* serology ( $S^+$ ) or positive anti-*Leishmania* DTH ( $DTH^+$ )). ELISA was performed with these sera using salivary gland sonicate of the sand fly *Lu. longipalpis*. FIG. 1A is a bar graph of anti-saliva IgG levels in individuals who converted from  $S^- \rightarrow S^+$  and those who converted from  $DTH^-$  to  $DTH^+$ . FIG. 1B is a bar graph of anti-saliva IgE levels in the individuals described in FIG. 1A. FIG. 1C is a

bar graph of anti-saliva IgG1 levels in the individuals described in FIG. 1A. FIG. 1D is a bar graph of anti-saliva IgG4 levels in the individuals described in FIG. 1A. The non-parametric paired Wilcoxon test was used to compare levels of anti-*Lu. longipalpis* saliva antibodies at time 0 and after 6 months. *P* value < 0.05 was established as the significance level.

5

**FIG. 2** is a set of two digital images and a bar graph showing salivary proteins recognized by Western blot analysis. FIGS. 2A and 2B are digital images of a Western blot of *Lu. longipalpis* salivary proteins reacted to human sera of individuals who converted from S<sup>-</sup> → S<sup>+</sup> to *Leishmania* (lanes 1–6) or from DTH<sup>-</sup> → DTH<sup>+</sup> to *Leishmania* (lanes 7–14). Symbols: –, time 0; +, 6 months.

10 FIG. 2C is a bar graph of the frequency of salivary proteins recognized by sera of 13 individuals who converted from DTH<sup>-</sup> → DTH<sup>+</sup> to *Leishmania*. The x-axis shows the different *Lu. longipalpis* salivary proteins (labeled by the approximate molecular weight) recognized by Western blot analysis, while the y-axis indicates the number of human sera recognizing a particular salivary protein.

15

#### SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the 20 accompanying sequence listing:

SEQ ID NO: 1 is the amino acid sequence of L JL34.  
SEQ ID NO: 2 is the nucleic acid sequence of L JL34.  
SEQ ID NO: 3 is the amino acid sequence of L JL18.  
SEQ ID NO: 4 is the nucleic acid sequence of L JL18.  
25 SEQ ID NO: 5 is the amino acid sequence of L JS193.  
SEQ ID NO: 6 is the nucleic acid sequence of L JS193.  
SEQ ID NO: 7 is the amino acid sequence of L JS201.  
SEQ ID NO: 8 is the nucleic acid sequence of L JS201.  
SEQ ID NO: 9 is the amino acid sequence of L JL13.  
30 SEQ ID NO: 10 is the nucleic acid sequence of L JL13.  
SEQ ID NO: 11 is the amino acid sequence of L JL23.  
SEQ ID NO: 12 is the nucleic acid sequence of L JL23.  
SEQ ID NO: 13 is the amino acid sequence of L JM10.  
SEQ ID NO: 14 is the nucleic acid sequence of L JM10.  
35 SEQ ID NO: 15 is the amino acid sequence of L JL143.  
SEQ ID NO: 16 is the nucleic acid sequence of L JL143.  
SEQ ID NO: 17 is the amino acid sequence of L JS142.  
SEQ ID NO: 18 is the nucleic acid sequence of L JS142.

SEQ ID NO: 19 is the amino acid sequence of LJL17.  
SEQ ID NO: 20 is the nucleic acid sequence of LJL17.  
SEQ ID NO: 21 is the amino acid sequence of LJM06.  
SEQ ID NO: 22 is the nucleic acid sequence of LJM06.  
5 SEQ ID NO: 23 is the amino acid sequence of LJM17.  
SEQ ID NO: 24 is the nucleic acid sequence of LJM17.  
SEQ ID NO: 25 is the amino acid sequence of LJL04.  
SEQ ID NO: 26 is the nucleic acid sequence of LJL04.  
10 SEQ ID NO: 27 is the amino acid sequence of LJM114.  
SEQ ID NO: 28 is the nucleic acid sequence of LJM114.  
SEQ ID NO: 29 is the amino acid sequence of LJM111.  
SEQ ID NO: 30 is the nucleic acid sequence of LJM111.  
SEQ ID NO: 31 is the amino acid sequence of LJM78.  
15 SEQ ID NO: 32 is the nucleic acid sequence of LJM78.  
SEQ ID NO: 33 is the amino acid sequence of LJS238.  
SEQ ID NO: 34 is the nucleic acid sequence of LJS238.  
SEQ ID NO: 35 is the amino acid sequence of LJS169.  
SEQ ID NO: 36 is the nucleic acid sequence of LJS169.  
SEQ ID NO: 37 is the amino acid sequence of LJL11.  
20 SEQ ID NO: 38 is the nucleic acid sequence of LJL11.  
SEQ ID NO: 39 is the amino acid sequence of LJL08.  
SEQ ID NO: 40 is the nucleic acid sequence of LJL08.  
SEQ ID NO: 41 is the amino acid sequence of LJS105.  
SEQ ID NO: 42 is the nucleic acid sequence of LJS105.  
25 SEQ ID NO: 43 is the amino acid sequence of LJL09.  
SEQ ID NO: 44 is the nucleic acid sequence of LJL09.  
SEQ ID NO: 45 is the amino acid sequence of LJL38.  
SEQ ID NO: 46 is the nucleic acid sequence of LJL38.  
SEQ ID NO: 47 is the amino acid sequence of LJM04.  
30 SEQ ID NO: 48 is the nucleic acid sequence of LJM04.  
SEQ ID NO: 49 is the amino acid sequence of LJM26.  
SEQ ID NO: 50 is the nucleic acid sequence of LJM26.  
SEQ ID NO: 51 is the amino acid sequence of LJS03.  
SEQ ID NO: 52 is the nucleic acid sequence of LJS03.  
35 SEQ ID NO: 53 is the amino acid sequence of LJS192.  
SEQ ID NO: 54 is the nucleic acid sequence of LJS192.  
SEQ ID NO: 55 is the amino acid sequence of LJM19.  
SEQ ID NO: 56 is the nucleic acid sequence of LJM19.  
SEQ ID NO: 57 is the amino acid sequence of LJL138.

SEQ ID NO: 58 is the nucleic acid sequence of LJL138.  
SEQ ID NO: 59 is the amino acid sequence of LJL15.  
SEQ ID NO: 60 is the nucleic acid sequence of LJL15.  
SEQ ID NO: 61 is the amino acid sequence of LJL91.  
5 SEQ ID NO: 62 is the nucleic acid sequence of LJL91.  
SEQ ID NO: 63 is the amino acid sequence of LJM11.  
SEQ ID NO: 64 is the nucleic acid sequence of LJM11.  
SEQ ID NO: 65 is the amino acid sequence of LJS138.  
SEQ ID NO: 66 is the nucleic acid sequence of LJS138.  
10 SEQ ID NO: 67 is the amino acid sequence of LJL124.  
SEQ ID NO: 68 is the nucleic acid sequence of LJL124.  
SEQ ID NO: 69 is the amino acid sequence of LJL35.  
SEQ ID NO: 70 is the nucleic acid sequence of LJL35.  
SEQ ID NO: 71 is an oligonucleotide primer.  
15 SEQ ID NO: 72 is an oligonucleotide primer.  
SEQ ID NO: 73 is an oligonucleotide primer.

#### DETAILED DESCRIPTION

20

##### *I. Abbreviations*

AAV	adeno-associated virus
AcNPV	Autographa California Nuclear Polyhedrosis Virus
25 alum	aluminum phosphate or aluminum hydroxide
BCG	Bacillus Calmette Guerin
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
CAV	canine adenovirus
30 CDR	complementarity determining region
CHV	canine herpes virus
CMV	cytomegalovirus
CTL	cytotoxic T lymphocyte
DMRIE	N-(2-hydroxyethyl)-N,N-diméthyl-2,3-bis(tetradecyloxy)-1- 35 propanammonium
DOPE	dioleoyl-phosphatidyl-ethanolamine
DTH	delayed type hypersensitivity
fMLP	N-formyl-methionyl-leucyl-phenylalanine
GM-CSF	granulocyte-macrophage colony stimulating factor
40 H	heavy chains

	HLB	hydrophile-lipophile balance
	ID	intradermal
	IM	intramuscular
	ISS	immunostimulating sequence
5	KLH	keyhole limpet hemocyanin
	L	light chains
	LB	Luria broth
	<i>Lu. longipalpis</i>	<i>Lutzomyia longipalpis</i>
	MVA	Modified Vaccinia virus Ankara
10	OFR	open reading frame
	<i>P. ariasi</i>	<i>Phlebotomus ariasi</i>
	PCR	polymerase chain reaction
	polyA	polyadenylation signal
	<i>P. papatasi</i>	<i>Phlebotomus papatasi</i>
15	PVDF	polyvinylidene difluoride
	SC	subcutaneous
	SCA	Single chain antibody
	sFv	single-chain antigen binding proteins
	SGH	salivary gland homogenate
20	SPGA	sucrose phosphate glutamate albumin
	tPA	tissue plasminogen activator
	V <sub>H</sub>	variable region of the heavy chain
	V <sub>L</sub>	variable region of the light chain
	VL	visceral leishmaniasis
25		

## II. Terms

Unless otherwise noted, technical terms are used according to conventional usage.

Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, 30 published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the disclosure, the following 35 explanations of specific terms are provided:

**Amplification of a nucleic acid molecule (for example, a DNA or RNA molecule):** A technique that increases the number of copies of a nucleic acid molecule in a specimen. An example of amplification is the polymerase chain reaction, in which a biological sample collected from a

subject is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of amplification may be characterized 5 by electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing using standard techniques. Other examples of amplification include strand displacement amplification, as disclosed in U.S. Patent No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Patent No. 6,033,881; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP 10 0320308; gap filling ligase chain reaction amplification, as disclosed in U.S. Patent No. 5,427,930; and NASBA<sup>TM</sup> RNA transcription-free amplification, as disclosed in U.S. Patent No. 6,025,134.

**Antibody:** immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, for instance, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen.

15 A naturally occurring antibody (for example, IgG, IgM, IgD) includes four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. However, it has been shown that the antigen-binding function of an antibody can be performed by fragments of a naturally occurring antibody. Thus, these antigen-binding fragments are also intended to be designated by the term "antibody." Specific, non-limiting examples of binding fragments 20 encompassed within the term antibody include (i) an Fab fragment consisting of the V<sub>L</sub>, V<sub>H</sub>, CL, and CH1 domains; (ii) an Fd fragment consisting of the V<sub>H</sub> and CH1 domains; (iii) an Fv fragment consisting of the V<sub>L</sub> and V<sub>H</sub> domains of a single arm of an antibody, (iv) a dAb fragment (Ward *et al.*, *Nature* 341:544-546, 1989) which consists of a V<sub>H</sub> domain; (v) an isolated complementarity determining region (CDR); and (vi) an F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab 25 fragments linked by a disulfide bridge at the hinge region.

Immunoglobulins and certain variants thereof are known and many have been prepared in recombinant cell culture (for example, see U.S. Patent No. 4,745,055; U.S. Patent No. 4,444,487; WO 88/03565; EP 0256654; EP 0120694; EP 0125023; Faoulkner *et al.*, *Nature* 298:286, 1982; Morrison, *J. Immunol.* 123:793, 1979; Morrison *et al.*, *Ann Rev. Immunol.* 2:239, 1984).

30 **Animal:** Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects, such as dogs.

**Conservative variants:** Conservative amino acid substitutions are those substitutions that do not substantially affect or decrease an activity or antigenicity of the *Lu. longipalpis* polypeptide.

35 Specific, non-limiting examples of a conservative substitution include the following examples:

	Original Residue	Conservative Substitutions
5	Ala	Ser
	Arg	Lys
	Asn	Gln, His
	Asp	Glu
	Cys	Ser
	Gln	Asn
10	Glu	Asp
	His	Asn; Gln
	Ile	Leu, Val
	Leu	Ile; Val
	Lys	Arg; Gln; Glu
	Met	Leu; Ile
15	Phe	Met; Leu; Tyr
	Ser	Thr
	Thr	Ser
	Trp	Tyr
	Tyr	Trp; Phe
	Val	Ile; Leu

The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibodies raised to the unsubstituted polypeptide 25 also essentially immunoreact with the substituted polypeptide, or that an immune response can be generated against the substituted polypeptide that is similar to the immune response against the unsubstituted polypeptide. Thus, in one embodiment, non-conservative substitutions are those that reduce an activity or antigenicity.

**cDNA (complementary DNA):** A piece of DNA lacking internal, non-coding segments 30 (introns) and expression control sequences. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

**Degenerate variant:** A polynucleotide encoding a *Lu. longipalpis* polypeptide that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are 35 included in the disclosure as long as the amino acid sequence of the *Lu. longipalpis* polypeptide encoded by the nucleotide sequence is unchanged.

**Delayed-type hypersensitivity (DTH):** An immune reaction in which T cell-dependent macrophage activation and inflammation cause tissue injury. A DTH reaction to the subcutaneous injection of antigen is often used as an assay for cell-mediated immunity.

**Epitope:** An antigenic determinant. These are particular chemical groups or peptide 40 sequences on a molecule that are antigenic, for instance, that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide. Specific, non-limiting examples of an epitope include a tetra- to penta-peptide sequence in a polypeptide, a tri- to penta-glycoside sequence in a polysaccharide. In the animal most antigens will present several or even 45 many antigenic determinants simultaneously. Such a polypeptide may also be qualified as an immunogenic polypeptide and the epitope may be identified as described further.

**Expression Control Sequences:** Nucleic acid sequences that control and regulate the expression of a nucleic acid sequence, such as a heterologous nucleic acid sequence, to which it is operably linked. Expression control sequences are operably linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, polyA signals, a start codon (for instance, ATG) in front of a protein-encoding polynucleotide sequence, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription of a nucleic acid. Promoters may be cell-type specific or tissue specific. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. Both constitutive and inducible promoters are included (see for example, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987).

For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac-hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (for example, metallothionein promoter) or from mammalian viruses (for example, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells. In one embodiment, the promoter is a cytomegalovirus promoter.

**Host cells:** Cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Also includes the cells of the subject.

**Immune response:** A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). The response can also be a non-specific response (not targeted specifically to salivary polypeptides) such as production of lymphokines. In one embodiment, an

immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a Th1 (a subset of helper T cells) response. In yet another embodiment, the response is a B cell response, and results in the production of specific antibodies.

5 **Immunogenic polypeptide:** A polypeptide which comprises an allele-specific motif, an epitope or other sequence such that the polypeptide will bind an MHC molecule and induce a cytotoxic T lymphocyte (“CTL”) response, and/or a B cell response (for example, antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide is derived.

10 In one embodiment, immunogenic polypeptides are identified using sequence motifs or other methods known in the art. Typically, algorithms are used to determine the “binding threshold” of polypeptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular substitution in a motif-15 containing polypeptide. Within the context of an immunogenic polypeptide, a “conserved residue” is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a polypeptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic polypeptide.

20 **Immunogenic composition:** A composition that, when administered to a subject induces an immune response to a *Lu. longipalpis* salivary polypeptide. In one embodiment, in particular a positive DTH response.

25 **Isolated:** An “isolated” biological component (such as a nucleic acid or protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been “isolated” include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant technology as well as chemical synthesis.

30 **Label:** A detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

35 **Leishmaniasis:** A parasitic disease spread by the bite of infected sand flies. The trypanosomatid parasite of the genus *Leishmania* is the etiological agent of a variety of disease manifestations, which are collectively known as leishmaniasis. Leishmaniasis is prevalent throughout the tropical and sub-tropical regions of Africa, Asia, the Mediterranean, Southern Europe (old world), and South and Central America (new world). The old world species are transmitted by the sand fly vector *Phlebotomus* sp. Humans, wild animals and domestic animals (such as dogs) are known to be targets of these sand flies and to act as reservoir hosts or to develop leishmaniasis.

Cutaneous leishmaniasis starts as single or multiple nodules that develop into ulcers in the skin at the site of the bite. The chiclero ulcer typically appears as a notch-like loss of tissue on the ear lobe. The incubation period ranges from days to months, even a year in some cases. The sores usually last months to a few years, with most cases healing on their own. The mucocutaneous type 5 can develop into erosive lesions in the nose, mouth, or throat and can lead to severe disfigurement. Visceral leishmaniasis often has fever occurring in a typical daily pattern, abdominal enlargement with pain, weakness, widespread swelling of lymph nodes, and weight loss, as well as superimposed infections because of a weakened immune system. Visceral leishmaniasis (VL) can result in high death rates. The onset of symptoms can be sudden, but more often tends to be insidious.

10 ***Lutzomyia longipalpis* (Lu. longipalpis):** A species of sand fly endogenous to the New World (South and Central America). This sand fly is the principal vector of American visceral leishmaniasis, a potentially fatal disease that primarily affects children in several countries of South and Central America.

15 **Lymphocytes:** A type of white blood cell that is involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

**Mammal:** This term includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

**Oligonucleotide:** A linear polynucleotide sequence of up to about 100 nucleotide bases in length.

20 **Open reading frame (ORF):** A nucleic acid sequence having a series of nucleotide triplets (codons), starting with a start codon and ending with a stop codon, coding for amino acids without any internal termination codons. These sequences are usually translatable into a polypeptide.

25 **Operably linked:** A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

30 **Polypeptide Modifications:** *Lu. longipalpis* polypeptides include synthetic embodiments of polypeptides described herein. In addition, analogues (non-peptide organic molecules), derivatives (chemically functionalized peptide molecules obtained starting with the disclosed polypeptide sequences) and variants (homologs) of these proteins can be utilized in the methods described herein. Each polypeptide of the disclosure is comprised of a sequence of amino acids, which may be either L-and/or D- amino acids, naturally occurring and otherwise.

35 Polypeptides may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified polypeptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C<sub>1</sub>-C<sub>16</sub> ester, or converted to an amide of formula NR<sub>1</sub>R<sub>2</sub> wherein R<sub>1</sub> and R<sub>2</sub> are

each independently H or C<sub>1</sub>-C<sub>16</sub> alkyl, or combined to form a heterocyclic ring, such as a 5- or 6-membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric, and other organic salts, or may be modified to C<sub>1</sub>-C<sub>16</sub> alkyl or 5 dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains may be converted to C<sub>1</sub>-C<sub>16</sub> alkoxy or to a C<sub>1</sub>-C<sub>16</sub> ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine, or iodine, or with C<sub>1</sub>-C<sub>16</sub> alkyl, C<sub>1</sub>-C<sub>16</sub> alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids.

10 Methylene groups of the peptide side chains can be extended to homologous C<sub>2</sub>-C<sub>4</sub> alkynes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this disclosure to select and provide conformational constraints to the structure that result in enhanced stability.

15 Peptidomimetic and organomimetic embodiments are envisioned, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid side chains, resulting in such peptido- and organomimetics of a *L. longipalpis* polypeptide having measurable or enhanced ability to generate an immune response. For computer modeling applications, a

20 pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs," Klegerman & Groves (eds.), 1993, *Pharmaceutical Biotechnology*, Interpharm Press: Buffalo Grove, IL, pp. 165-174 and *Principles of Pharmacology*

25 Munson (ed.) 1995, Ch. 102, for descriptions of techniques used in CADD. Also included are mimetics prepared using such techniques.

**Pharmaceutically acceptable vehicles or excipients:** The pharmaceutically acceptable vehicles or excipients of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and 30 formulations suitable for pharmaceutical delivery of the polypeptides, plasmids, viral vectors herein disclosed.

In general, the nature of the vehicle or excipient will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, 35 physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, freeze-dried pastille, powder, pill, tablet, or capsule forms), conventional non-toxic solid vehicles or excipients can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral vehicles or excipients, immunogenic compositions to be administered can contain minor amounts of non-toxic

auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

***Phlebotomus ariasi (P. ariasi):*** A species of *Phlebotomus* (sand flies) genus endogenous to the Old World, in particular to southern Europe and Mediterranean countries, more particularly to 5 Spain and France. This sand fly is a proven vector of visceral leishmaniasis. *P. ariasi* is a member of the subgenera of *Phlebotomus Larroussius*.

***Phlebotomus perniciosus (P. perniciosus):*** A species of *Phlebotomus* (sand flies) genus endogenous to the Old World, in particular to southern Europe, and Mediterranean countries, more particularly to France, Italy, Greece, Morocco, and Spain. This sand fly is a proven vector of the 10 visceral leishmaniasis. *P. perniciosus* is a member of the subgenera of *Phlebotomus Larroussius*.

**Polynucleotide:** The term polynucleotide or nucleic acid sequence refers to a polymeric form of nucleotide at least 10 bases in length, thus including oligonucleotides and genes. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both 15 of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (for example, a cDNA) independent of other sequences. The polynucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term 20 includes single -and double -stranded forms of DNA.

**Polypeptide:** Any chain of amino acids, regardless of length (thus encompassing oligopeptides, peptides, and proteins) or post-translational modification (for example, glycosylation, phosphorylation, or acylation). A polypeptide encompasses also the precursor, as well as the mature protein. In one embodiment, the polypeptide is a polypeptide isolated from *Lu. longipalpis*, or 25 encoded by a nucleic acid isolated from *Lu. longipalpis*, such as the *Lu. longipalpis* polypeptides disclosed herein.

**Probes and primers:** A probe comprises an isolated polynucleotide attached to a detectable label or reporter molecule. Primers are short polynucleotides. In one embodiment, polynucleotides are 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA 30 strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, for example, by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art. One of skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for 35 example, a primer comprising 20 consecutive nucleotides will anneal to a target with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, probes and primers may be selected that comprise at least 15, 20, 25, 30, 35, 40, 50 or more consecutive nucleotides.

**5** **Protein Purification:** The *Lu. longipalpis* polypeptides disclosed herein can be purified by any of the means known in the art. See, for example, *Guide to Protein Purification*, Deutscher (ed.), *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982. Substantial purification denotes purification from other proteins or cellular components. A substantially purified protein is at least 60%, 70%, 80%, 90%, 95%, or 98% pure. Thus, in one specific, non-limiting example, a substantially purified protein is 90% free of other proteins or cellular components.

**10** **Purified:** The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide preparation is one in which the polypeptide is more enriched than the polypeptide is in its natural environment. A polypeptide preparation is substantially purified such that the polypeptide represents several embodiments at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, of the total polypeptide content of the preparation. The same applies for polynucleotides. The polypeptides disclosed herein can be purified by any of the means known in the art (see, for example, *Guide to Protein Purification*, Deutscher (ed.), *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982).

**20** **Recombinant:** A recombinant polynucleotide is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. In one embodiment, a recombinant polynucleotide encodes a fusion protein.

**25** **Selectively hybridize:** Hybridization under moderately or highly stringent conditions that excludes non-related nucleotide sequences.

**30** In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (for example, GC v. AT content), and nucleic acid type (for example, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

**35** A specific, non-limiting example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). One of skill in the art can readily determine variations on these conditions (for example, *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). The hybridization conditions can be carried out over 2 to 16 hours. Washing can be carried out using only one of the above conditions, for example, high stringency

conditions, or each of the conditions can be used, for example, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

5       **Sequence identity:** The similarity between amino acid sequences is expressed in terms of the percentage identity between the sequences. The higher the percentage, the more similar the two sequences are. Homologs or variants of a *Lu. longipalpis* polypeptide will possess a relatively significant high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various  
10 programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482,  
1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci.*  
USA 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989;  
Corpet *et al.*, *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci.*  
USA 85:2444, 1988. Altschul *et al.*, *Nature Genet.* 6:119, 1994 presents a detailed consideration of  
15 sequence alignment methods and identity calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.*  
215:403, 1990) is available from several sources, including the National Center for Biotechnology  
Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence  
analysis programs blastp, blastn, blastx, tblastn, and tblastx. A description of how to determine  
20 sequence identity using this program is available on the NCBI website on the internet.

Homologs and variants of a *Lu. longipalpis* polypeptide are typically characterized by  
possession of at least 75%, for example at least 80%, sequence identity counted over the full length  
alignment with the amino acid sequence of the *Lu. longipalpis* polypeptide using the NCBI Blast 2.0,  
25 gapped blastp set to default parameters. The comparison between the sequences is made over the full  
length alignment with the amino acid sequence given in this present disclosure, employing the Blast 2  
sequences function using the default BLOSUM62 matrix set to default parameters, (gap existence cost  
of 11, and a per residue gap cost of 1).

When aligning short peptides (fewer than around 30 amino acids), the alignment should be  
performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters  
30 (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences  
will show increasing percentage identities when assessed by this method, such as at least 80%, at least  
85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the  
entire sequence is being compared for sequence identity, homologues and, variants will typically  
possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess  
35 sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference  
sequence. Methods for determining sequence identity over such short windows are available at the  
NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges  
are provided for guidance only; it is entirely possible that strongly significant homologues could be  
obtained that fall outside of the ranges provided.

**Specific binding agent:** An agent that binds substantially only to a defined target. Thus a *Lu. longipalpis* specific binding agent is an agent that binds substantially to a *Lu. longipalpis* polypeptide.

In one embodiment, the specific binding agent is a monoclonal or polyclonal antibody that specifically binds the *Lu. longipalpis* polypeptide.

5       **Subject:** Living multi-cellular vertebrate organisms, a category that includes both human veterinary subjects, including human and non-human mammals. In one embodiment, the subject is a member of the canine family, such as a dog. In another embodiment, the subject is a human.

10      **T Cell:** A white blood cell critical to the immune response. T cells include, but are not limited to, CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells. A CD4<sup>+</sup> T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. CD8<sup>+</sup> T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a CD8 T cells is a cytotoxic T lymphocytes. In another embodiment, a CD8 cell is a suppressor T cell.

15      **Therapeutically active polypeptide:** An agent, such as a *Lu. longipalpis* polypeptide, that causes induction of an immune response, as measured by clinical response (for example, increase in a population of immune cells, production of antibody that specifically binds the *Lu. longipalpis* polypeptide, a measurable reduction in symptoms resulting from exposure to *Leishmania*, or protection from infection with *Leishmania*). Therapeutically active molecules can also be made from nucleic acids. Examples of a nucleic acid based therapeutically active molecule is a nucleic acid sequence that encodes a *Lu. longipalpis* polypeptide, wherein the nucleic acid sequence is operably linked to a control element such as a promoter. Therapeutically active agents can also include organic or other chemical compounds that mimic the effects of the *Lu. longipalpis* polypeptide.

20      The terms "therapeutically effective fragment of a *Lu. longipalpis* polypeptide" includes any fragment of the *Lu. longipalpis* polypeptide, or variant of the *Lu. longipalpis* polypeptide, or fusion protein including a *Lu. longipalpis* polypeptide, that retains a function of the *Lu. longipalpis* polypeptide (such as immunogenicity), or retains the ability to reduce the symptoms from exposure to *Leishmania*, or to protect from infection with *Leishmania*.

25      Thus, in one embodiment, a therapeutically effective amount of a fragment of *Lu. longipalpis* polypeptide is an amount used to generate an immune response to the polypeptide. In another embodiment, a therapeutically effective amount of a fragment of a *Lu. longipalpis* polypeptide is an amount of use to prevent or treat a *Leishmania* infection in a subject. Treatment refers to a therapeutic intervention that confers resistance to infection with *Leishmania*, or a reduction in the symptoms associated with exposure to *Leishmania*. Specific, non-limiting examples of a polypeptide fragment are the N-terminal half or the C-terminal half of one of the *P. Lu. longipalpis* polypeptide disclosed herein. It should be noted that fusion proteins are included, such as a fusion with six histidine residues, a c-myc tag, or any other polypeptide tag. Such fusions are known to one of skill in the art, and are often used in protein purification.

30      **Transduced:** A transduced cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transduction encompasses all

techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

5 **Vector:** A nucleic acid molecule as introduced into a host cell, thereby producing a transduced host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

10 **Vaccine:** Composition that when administered to a subject, induces a decrease of the severity of the symptoms of a disorder or disease. In one embodiment, a vaccine decreases the severity of the symptoms of leishmaniasis and/or decreases the parasitic load.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. "Comprise" means "include," and a composition that comprises a polypeptide includes that polypeptide. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for polynucleotides or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

25

#### *Lu. longipalpis* Polynucleotides and Polypeptides

Salivary polypeptides from sand fly species *Lu. longipalpis*, are disclosed herein.

30 LJL34 (SEQ ID NO: 1)

MLQIKHLLIFVGLL VVVNAQSNYCKQESCSSGGVERPHIGCKNSGDFSETCSGDAEIVKMDK  
KKQNLLVKMHNRLDRFARGAVPGFAPAAKMPMLKWNLDELAKLAEYNVRTCKFAHDKC  
RAIDVCPYAGQNLAQMMMSYPTHRDLNYVLKNLTREWFWEYRWAKQLSQLDNYVGGPGKD  
NKQIGHFTAFVHEKTDKVGCAIARFTNEHNFKETLLACNYCYTNMMKERIYTQGKPCSQCQ  
35 SKKCGPVYKNLCDPSEKVDPTPDVLKQWKHGK

LJL18 (SEQ ID NO:3)

MLLRSLFVLFLIFL TFCNAEEELIERKLTGKTIYISTIKLPWFQALNHCVKNGYTMVSIKTFFEE  
NKELLKELKRVIRTEDTQVWIGGLKHHQFANFRWVSDGSHVATASGYTNWAPGEPADSFY

40 YDQFCMAMLFRKDGA PWDDLNCWVKNLFVCEKRDD

LJS193 (SEQ ID NO:5)

MKLLQIIFSLFLVFFFPTSNGALTGNESAANAAPLPVVLWHGMGDSCCFPFSLGSIKKLIQQIP  
GIHVVSLSKIGKSLIEDYESGFFVHPDKQIQEVCESLQNDTLANGNAIGFSQGSQFLRGLVQR  
5 CSSIQVRNLISIGGQHQGVFGLPYCPSLSRKTCEYFRKLLNYAAYEKWVQKLLVQATYWH  
PLNEDAYRTGSTFLADINNERQINNDYINNIRKLNRFVMVKFLNDSMVQPIESSFFGYAPGT  
DTEVLPLKQSKIYLEDRGLQSVPIDYLECGGDHLQFTKEWFIKFIIIPYIQLQ

LJS201 (SEQ ID NO: 7)

10 MRNFAVVS LA VAVLLFC AW PINAEDNEEV GKAREK RGLKDAMEH FKNGF KELTKDFKLPS  
LPSLPGFGKKPESGSSEDGDKTEDTSGSKDDQSKDNTVEES

LJL13 (SEQ ID NO: 9)

15 MNFLLKIFSLLCLCGLGYSWQDVRNADQTLWAYRSCQKNPEDKDHVPQWRKFELPDDEKT  
HCYVKCVWTRLGAYNENENVFKIDVITKQFNERGLEVPAGLDQELGGSTDGTCKAVYDKS  
MKFFKSHFMDFRNAYYATYDGSDEWFSKNPDVKPKGTKVSEYCKNKDDGDCKHSCSMYY  
YRLIDEDNLVIPFSNLPDYPEDKLEECRNEAKSANECKSSVIYQCLENADKSALDASLNIL  
DEFSGRY

20 LJL23 (SEQ ID NO: 11)

MFLKWVVC AFATVFLGV SQA APPGVEWYHFG LIADM DKK SIASDK TTFNSVL KIDE LRHN  
TKTDQIYVRSRVKKPVSTRYGFKGRGA ELSEIVV FNN KLYTVDDKSGITFRITKD GKL FPW  
VILA DADGQRPDGFKGEWATIKDDTIYVGSTGMLKFTSSLWVKKITKDGVVTSHDWTDKY  
RKILKALNMPNGFVWHEAVTWSPFRKQWVFM PRKCSRHPFSQELEERTGCNKIVTADENF  
25 DIQVIHQDQPYNLASGFSSFRFIPGKNERLLALRTVEQEDQVKTWAVVMDMKGTVL MYE  
KELYDEKFEGLAFFGGIKKN

LJM10 (SEQ ID NO: 13)

30 MALKFLPVLLSCFAMSTALQVTEKELSDGKKIFISKVELNWFEALDFCIHRLTLLSIKSAK  
ENV DVTKAIRAE LFDSKKLAHVWTGGIRHSQDKYFRWINDGTKVVKRVYTNWFTGEPNN  
GYWKDEF CLEIYYKTEEGKWNDDKCHVHHFVCQEKK

LJL143 (SEQ ID NO: 15)

35 MNSINFLSIVGLISFGFIVAVKCDGDEYFIGKYKEKDET LFFAS YGLKRDPCQIVLGYKCSNN  
QTHFVLNFKTNKKSCISA IKLTSYPKINQNSDLTKNLYCQTGGIGTDNC KLVFKKRKRQIAAN  
IEIYGIPAKKCSFKDRYIGADPLHVDSYGLPYQFDQEHGW NVERYNIFKDTRFSTEVFYHKN  
GLFNTQITYLAEEDSFSEAREITAKDIKKFSIILPNEEYKRISFLD VYWFQETMRKKPKY PYIH  
YNGECSNENKTCELVFDTDELMTYALVKVFTNPESDGSR LKEEDLGRG

LJS142 (SEQ ID NO: 17)

MAFSNTLFVLFVSLFTFCGADQTLIEKELTGRTVYISKIKLNWNDAYCIRNGLTFAKIKSA  
EENTELSEKLKTVIRTEEFQVWIGGIEHHQDSSFRVVSQDNPITNKLGYKYTNWNTGEPTNY  
QNNEYCLEILFRKEDGKWNDPCSARHHFVCEKRTK

5

LJL17 (SEQ ID NO: 19)

MQNFLLVSLALAALMLCAEAKPYDFPLYQDLIQGVIQRESQAEREKRSPNEDYEKQFGDIVD  
QIKEISFNVMKMPHFGSSDDNRDDGEYVDHHYGDEDDRDYDHY

10 LJM06 (SEQ ID NO: 21)

MKFYIFGVFLVSLALCNAEDYDKVKLTGRTVYISRSKAPWFTALDNCNR  
RFTFAMIKSQKENEELTNALLSVIKSDEENVWIGGLRHDLDDYFRWISFGTALSCTSNTWA  
PKEPTGRPHRTQNDEFQCMQMSFKDGGKWSDNTCWRKRLYVCEKRD

15 LJM17 (SEQ ID NO: 23)

MRFFFVFLAIVLFQGIHGAYVEIGYSLRNITFDGLDTDDYNPKFNIPTGLAVDPEGYRLFIAIPR  
RKPKVPTYVAELNMVMNPFPVERAPSFEKFKFNGEKKDLNVYQPVIDDCRRLWVLDI  
GKVEYTGGADQYPKGKPTLIAYDLKKDHTPEIHRFEIPDDLYSSQVEFGFAVDVVNTKG  
DCTESFVYLTNFKDNSLIVYDETQKKAWKFTDKTFEADKESTFSYSGEEQMKYKVGLFGIAL  
20 GDRDEMGRHPACYIAGSSTKVYSVNTKELKTENGQLNPQLHGDRGKYTDAIALAYDPEHK  
VLYFAESDSRQVSCWNVNMLKPNDTDFVFSARFTFGTDILVDSKGMLWIMANGHPPVED  
QEKIWKMRFVNRKIRIMKVDTERVFYSRCNPNYKPPK  
EIEV

25 LJL04 (SEQ ID NO: 25)

MIKEVFLALLVALAQCANEIPINRQGKDYPVPIIDPNKSSSDYFDDRFYPDIDDEGIAEAPK  
DNRGKSRGGAAGAREGRLGTNGAKPGQGGTRPGQGGTRPGQGGTRPGQGGTRPGQGGT  
RPGQGRTKPAQGTTTQAQGTRNPGSGVTKEAQDASKQGQGKRRPGQVGGKRPQANAPNA  
GTRKQQKGSRGVGRPDLSRYKDAPAKFVFKSPDFSGEGKPTVNYFRTKKKEHIVTRGSPN  
30 DEFVLEILDGDPTGLGLKSETIGKDTRLVLENPNGNSIVARVKIYKNGYSG

LJM114 (SEQ ID NO: 27)

MNSVNTLILTLLFAIFLLVKRSQAFLPSDPSICVKNLVLDTGRTEESEYFPDIKNVKNGKRVY  
IVCTDSDAVDYKFYICFDMNRSLGPPYPEEEILRESTVTYAQIYELMTTETTETKKPKKKPKN  
35 SKTDDPPAIRPGFSRNSISV

LJM111 (SEQ ID NO: 29)

MKLFFFYTFGLVQTIFGVEIKQGFKWNKILYEGDTSENFPDNNILTAFAYDPESQKLFLTV  
PRKYPETMYTLAEVDTEKNSFESGDTSPLLGKFGHETGKELTSVYQPVIDECHRLWVVDVG

-20-

SVERNSDGTEGQPEHNPTLVAYDLKEANYPEVIRYTFPDNSIEKPTFLGGFADVVKPDECSE  
TFVYITNFLTNALIVYDHKNKDSWTVQDSTFGPDKSKFDHDGQQYEYEAGIFGITLGERDN  
EGNRQAYYLVASSTKLHSINTKELKQKGSKVNANYLGDRGESTDAIGLVYDPKTKTIFFVES  
NSKRVSCWNTQETLNKDKIDVIYHNADFSFGTDISIDSQDNLWFLANGLPPLENSDKFVFTKP  
5 RYQIFKVNIQEAIAGTKCEKNL

LJM78 (SEQ ID NO: 31)  
MTFLIILGAFLLVQIITASALGLPEQFKGLEDPKKPLAETYHEGLNDGKTDEMVDIFKSLSD  
EFKFSDENLDVGEEKNYKKRDITQNSVARNFLSNKGIPSMPSLPSMPSMPSIPSLWSSQTQA  
10 APNTALALPESDYSLLDMPNIVKNFLKETRDLYNDVGAFLKAITEALTNRSSSSQLLSPMVS  
TNKTKEFIRNEIQKVRKVRNFVQETLQKIRDISAAIAKKVKSSECLSNLTDIKGLVSDGINCLK  
EKFNDGKRILQLYNNNLLKGLKIPNDLMVELKKCDTNQNNTLGRIICYFLTPLQLEKEQILLPV  
EFIKRILELTHYFSTMKEDLINCIGITIASIT

15 LJS238 (SEQ ID NO:33)  
MLKIVLFLSVLAVLVICVAAMPGSNVPWHISREELEKLREARKNHKALEKAIDEIDLKYL

LJS169 (SEQ ID NO:35)  
MKFSCPVFVAIFLLCGFYRVEGSSQCEEDLKEEAEAFFKDCNEAKANPGEYENLTKEEMFEE  
20 LKEYGVADTDMETVYKLVEECWNELETTDCKRFLEEAECFKKNICKYFPDEVKLKKK

LJL11 (SEQ ID NO: 37)  
MLFFLNFFVLVFSIELALLTASAAAEDGSYEIIIILHTNDMHARFDQTNAGSNKCQEKDIAASK  
CYGGFARVSTMVKKFREENGSSVLFNAGDTYTGTPWFTLYKETIATEMMNILRPDAASLG  
25 NHEFDKGVEGLVPFLNGVTFPILTANLDTSQEPTMTNAKNLKRSMIFTVSGHRVGVIGYLTP  
DTKFLSDVGKVNFIVEVEAINTEAQRLKKEENAEIIVVGHSGLIKDREIAEKCPLVDIIVGGHS  
HTFLYTGSQPDREVVPDVYPVVVTQSSGKKVPIVQAYCFTKYLGYFKVTINGKGNVVGWTG  
QPIILLNNNIPQDQEVLTALEYRERVENYGNRIVGSRVILNGGHTECRFHECNMGNLITDA  
FVYANVISTPMSTNAWTDASVLYQSGGIRAPIDPRTAAGSITRLELDNVLPFGNALYVVKV  
30 PGNVLRKALEHSVHRYSNNTSGWGEFPQVSGLKIRFNVNEEIGKRVKSVKVLCSNCQPEYQP  
LRNKKTYNVIMDSFMKDGGDGYSMFKPLKIIKTLPLGDIETVEAYIEKMGPIFPAVEGRITV  
LGGLQKSDEDWH

LJL08 (SEQ ID NO: 39)  
35 MKQILLISLVVILAVLAFNVAEGCDATCQFRKAIEDCKKADNSDVLQTSVQTTATFTSMDT  
SQLPGNNVFKACMKEAKEFRAGK

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LJS105 (SEQ ID NO: 41)

MNVLFVSFTLTILLLCVKARPEDFVALQDQANFQKCLEQYPEPNQSGEVLACLKKREGAKD  
FREKRSLDDIEGTFQESGN

LWGA

5

LJL09 (SEQ ID NO: 43)

MKITVILFTGFTIALVSSAVLKKNGETIEEEEVRAEQLRREINEELDRRNINTVAAWAYASNI  
TEVNLKNMNDVSVETAKYYKELASELKGFAKEYKSEDLKRQIKKLSKLGYSALPSEKYKE  
LLEAITWMESNYAKVKVCSYKDPKKCDLALEPEITEILIKSRDPEELKYYWKWYDKAGTP  
10 TRESFNKYVQLNREAALDGFGYSGAESWLDEYEDETFEKQLEDIFAQIRPLYEQLHAYVRFK  
LREKYGNDVVSEKGPIPMMHLLGNMWGQTWSEVAPILPVYYPEKKLLDVTDEMVKQGYTPIS  
MFEKGDEFFQSLNMTKLPKTFWEYSILEKPQDGRELICHASAWDFYTKDDVRKQCTRVTMD  
QFFTAHHELGHIQYYLQYQHLPsvYREGANPGFHEAVGDVLSLSVSSPKHLEKVGLLDFKF  
DEESQINQLLNLAQKMAFLPFAYTIDKYRWGVFRGEISPSEYNCKFWEMRSYYGGIEPPIAR  
15 SESDFDPPAKYHISSDVEYLRYLVSFIQFQFHQAVCQKTGQFVNDPEKTLNCIDYQSAEA  
GNAFKEMLKLGSQPKWPDAMEILTGQRKMDASALIEYFRPLSEWLQKKNKELGAYVGWDK  
STKCVKNVS

LJL38 (SEQ ID NO: 45)

20 MKTFALIFLALAVFVLCIDGAPTFVNLLDDVQEEVEVNTYEP

LJM04 (SEQ ID NO:47)

MNHLCFIIIALFFLVQQSLAEHPEEKCIRELARTDENCILHCTYSYYGFVDKNFRIAKKHVQKF  
KKILVTGAVPKKEKK  
25 KLLEHIEACADSANADQPQTKDEKCTKINKYYRCVVDGKILPWNSYADAIIKFDKTLNV

LJM26 (SEQ ID NO: 49)

MKIIFLAAFLADGIWAAEEPSVEIVTPQSVRRHATPKAQDARVGSESATTAPRSESMDYW  
ENDDFVFPFEGPKDGFEDWNLKIVFEENGNAILSPLSVKLLMSLLFEASASGTLTQHQLR  
30 QATPTIVTHYQSREFYKNIFDGLKKKSNDYTVHFGTRIYVDQFVTPRQRYAAILEKHYLTDL  
KVEDFSKAKETTQAINSWSNITNEHIKDLVKEEDVQNSVMLMLNAVYFRGLWRKPFNRTL  
PLPFHVSADESKTTDFMLTDGLYYFYEAKELEDAKILRIPYKGKQYAMTVILPNSKSGIDSFVR  
QINTVLLHRIKWLMDEVECRVILPKFHDMTNELKESLVKLGISQIFTSEASLPSLARGQGVQ  
NRLQVSNVIQKAGIIVDEKGSTAYAASEVSLVNKGDEFVMFNAHPFLFTIEDETTGAILF  
35 TGKVVDPQTQ

LJS03 (SEQ ID NO: 51)

MRFLLLAFSVALVLSPTFAKPGLWDIVTGINDMVKNTANALKNRLTTSVTLFTNTITEAIKNA  
NSSVSELLQQVNETLTDIINGVGQVQSAFVNSAGNVVVQIVDAAGNVLEVVEAGNIVEV  
AGTALETIPLPGVVIQKIIDALQGNAGTTSDSASSTVPQQS

5

LJS192 (SEQ ID NO: 53)

MVKYSCLVLVAIFLLAGPYGVVGSCENDTEAAKYLQDECNAGEIADEFLPFSEEEVGEALS  
DKPENVQEVTNIVRGCFEAEQAKEHGKcerfsalsqcyieknlcqff

10 LJM19 (SEQ ID NO: 55)

MKFFYLIFSAIFFLADPALVKCSEDCEIFHDNAYLLKLDCEAGRVPVEYDDISDEEIYEITV  
DVGVSSDQEKVAKIIRECIAQVSTQDCTKFSEIYDCYMKKICNYYPENM

LJL138 (SEQ ID NO: 57)

15 MHLQLNLCAILSVLNGIQGAPKSINSKSCAISFPENVTAKKEPVYLKPSNDGSLSTPLQPSGP  
FVSLKIGESLAIFCPGDGKDVTITCNFDLASYSCKNSTDTIETEEVCGGSGKVKVVGFP  
LPSGNFHSIYQTCFDKKNLTPLYSIHILNGQA  
VGYHLKHTRGSFRTNGIYGVNIDKLYKTQIEKFNLFGPKQTFRRPLNFLSRGHLSPFVDF  
TFRREQHATEMYINTAPQYQSIQNQGNWLRVENHVRDLAKVLQKDITVVTGILGILRLKSKKI  
20 EKEIYLGDDVIAVPAMFWKAVFDPQKQEAIVFVSSNNPHVKTTFNPNCVDCAQAGFNDNL  
EYFSNYSIGLTICCKLEEFVKRNIILPKEVNNKNYTKLLKFPKTRNKEGDKVVRKRAKG  
A

LJL15 (SEQ ID NO: 59)

25 MNLHLAIILFVSYFTLITATDLIEKELSDCKKIFISKAELTWFQALDFCTEQNLTLLSIKSAREN  
DEVTKAVRAEVHLPDTKSHIWLGGIRYDQDKDFRWISDGTIVTAKTVYINWYQGEPENGGR  
YQKEFCMELYFKTPAGQWNDDICTAKHHFICQEKK

LJL91 (SEQ ID NO: 61)

30 MNLPLAIILFVSYFTLITAADLTEKELSDGKKIFISKAELSWFDALDACTEKDLTLTIKSAREN  
EEVTKAVERAECVHLPDTKSHIWLGGIRYDQDKDFRWISDGTIVTAKTVYINWYQGEPENGGR  
QKEFCMELYFKTPAGQWNDDICTAKHHFICQEKK

LJM11 (SEQ ID NO: 63)

35 MKVFFSIFTLVLFGQTLGADTQGYKWKQLLYNNVPGSYNPDMISTAFAYDAEGEKLFLA  
VPRKLPRVPYTLAEVDTKNSLGVKGKHSPLLNKFSGHKTGKELTSIYQPVIDDCRRLWVVDI  
GSVEYRSRGAKDYPSPHRPAIVAYDLKQPNYPEVVRYYFPTRLVEKPTYFGGFAVDVANPKG  
DCSETFVYITNFLRGALFIYDHKKQDSWNVTHPTKAERPTKFDYGGKEYEFKAGIFGITLGD  
RDSEGNRPAYYLAGSAIKVYSVNTKELKQKGGKLNPELLGNGKYNDAIALAYDPKTKVIF

FAEANTKQVSCWNTQKMPLRMKNTDVYTSSRFVFGTDISVDSKGGLWFMSNGFPPIRKSE  
KFKYDFPRYRLMRIMDTQEAIAGTACDMNA

LJS138 (SEQ ID NO: 65)

5 MQSKILSFVLFTSLGYVLGETCSNAVKKGATSYSTTDATIVSQIAFVTEFSLECSNPGSEKISL  
FAEVDGKIPVAMIGDTTYQVSWNEEVNKARSGDYSVKLYDEEGYGAVRKAQRSGEENKV  
KPLATVVVRHPGTYTGPWFNSEILAAGLIAVVAYFAFSTRSKILS

LJL124 (SEQ ID NO: 67)

10 MVSILLISLILNLLVFYAKARPLEDISSDLSPDYYITEGYDGVKEKREIELVPVTFGIFNIHTTPA  
PRITFEW

LJL35 (SEQ ID NO: 69)

15 MKLFCLIFVVVFVALEVCIETVKAMEATEEISVKLQDDANEPPDSLDLDEGLPADFEDYNNQ  
AEYKPNPRGDYRRR

In one embodiment, a polypeptide including SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 is disclosed herein. Homologous polypeptides having an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, 20 SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 are disclosed herein. Fusion proteins including SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, 25 SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 are also disclosed herein.

35 Fragments and variants of the *Lu. longipalpis* polypeptides identified above are disclosed herein and can readily be prepared by one of skill in the art using molecular techniques. In one embodiment, a fragment of a *Lu. longipalpis* polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids of a *Lu. longipalpis* polypeptide. In another embodiment, a fragment of a *Lu.*

*longipalpis* polypeptide includes a specific antigenic epitope found on a full-length *Lu. longipalpis* polypeptide.

In one embodiment, a fragment is at least 19 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids in length from any polypeptide (including polypeptides as given in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 conservative variants thereof, and homologues thereof), or any fragment that retains at least an epitope.

Fusion proteins including a *Lu. longipalpis* polypeptide can also be produced using methods known to one of skill in the art. In one embodiment, a fusion protein includes an amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or a conservative variants thereof, and a marker polypeptide. Marker polypeptides include, but are not limited to, polypeptide tags, such as a polypeptide to aid in protein purification (for example, six histidine residues or c-myc polypeptide), or an enzymatic marker (for example, alkaline phosphatase), or a fluorescent maker (for example, green fluorescent protein).

One skilled in the art, given the disclosure herein, can purify a *Lu. longipalpis* polypeptide using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the *Lu. longipalpis* polypeptide can also be determined by amino-terminal amino acid sequence analysis.

Minor modifications of the *Lu. longipalpis* polypeptide primary amino acid sequences may result in peptides which have substantially equivalent activity as compared to the unmodified counterpart polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein.

Polynucleotides encoding salivary polypeptides from *Lu. longipalpis* sand fly are disclosed herein, such as polynucleotides encoding SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67.

Specific, non-limiting examples of *Lu. longipalpis* nucleic acid sequences include SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:18, SEQ ID

NO:20, SEQ ID NO:22, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, or SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and degenerate variants thereof. These 5 polynucleotides include DNA, cDNA, and RNA sequences that encode a *Lu. longipalpis* polypeptide. It is understood that all polynucleotides encoding a *Lu. longipalpis* polypeptide are also included herein, as long as they encode a polypeptide with the recognized activity, such as the binding to an antibody that recognizes the polypeptide, the induction of an immune response to the polypeptide, or an effect on survival of *Leishmania* when administered to a subject having leishmaniasis or who 10 undergoes a decrease in a sign or a symptom of *Leishmania* infection.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the *Lu. longipalpis* polypeptide encoded by the nucleotide sequence is functionally 15 unchanged.

Specific, non-limiting examples of a polynucleotide encoding a *P. ariasi* polypeptide are set forth below:

LJL34 (SEQ ID NO:2)

20 AGTTGTGGAGCTTGGTCATTTACGTGATGTTGCAAATTAAACATCTTCTGATTGGTGGGATTGCTCGTGGTTAATGCACAGAGCAATTACTGCAAACAGGAATCGTGCCTCATCGGGAGGTGTTGAGAGACCCATATTGGGTGCAAAACTCTGGAGATTGGTCCGAAACTTGCTCCGGAGATGCAGAAATTGTTAAGATGGACAAGAAGAAGCAGAACCTCCTGTGAAATGCACAATGCCTGAGAGATAGATTGCTCGTGGTGCACTGCCAGGTTGCACCAAGTGCAGAAATGCAATGCTAACTTGCCACGATGCTCTGCCCCTATGCTGGACAGAAATCTAGCTCAAATGATGTCCTATCCTACCCATCGAGATCTAAACTATGTTCTTAAAGAATCTCACAAGGGAATGGTCTGGGAGTACAGATGGCTAAGCAATCTCAGCTTGAATAATTACGTGGGTGGCCTGGGAAAGACAACAAACAAATTGGACATTTCACAGCTTTGTTGCATGAGAAAACAGACAAAGTGGATGCGCTATAGCTCGATTACAATGAGCACAATTTCAGGAGACCCTCCTAGCTGCAACTACTGCTACACGAATATGATGAAGGAGAGGATCTACACGCAGGGAAAACCTTGTTCACAGTGTCAAGCAGAAAAGTGTGGGCCAGTCTACAGAACCTGTGATCCTCGGAGAAGGTTGATCCAACCTCCTGATGTCCTTAAGCAATGGAAAGCATGGAAAATGATTATTAAGCTCACTCAAATGTTCCAATCCAAAAAA

35 AAAAAAAAAAAAAAA

LJL18 (SEQ ID NO:4)

TTTGAGAAAACATTCCCTGTGAGTTAAATAGTGGTAAATTAAATCAAGAGAATGTTGCTTCGTTCTGTTCTTCTAATTCTAACATTCTGCAACGCTGAGGAAGAA

CTTATTGAGAGAAAGTTAACAGGAAAAACGATCTATCTCAACAATAAAGCTCCGTG  
 GTTCCAAGCTCTTAATCATTGTGTTAAAATGGCTACACAATGGTGTCAATTAAAGACATT  
 TGAAGAGAATAAAGAACTCCTAAAGAACTCAAAAGGGTATTAGGACAGAAAGATACA  
 CAAGTTGGATTGGAGGCCTCAAACATCATCAATTGCAAACCTTCGTTGGTAAGCGAT  
 5 GGAAGGCCACGTAGCAACAGCTCAGGGTACACCAATTGGGCCAGGGAGCCAGCTG  
 ATTCCTTCTATTACGATCAATTGCAATGGCGATGTTGTTAGAAAAGACGGCGCTCCGT  
 GGGATGATTGAATTGTTGGGTTAAGAATCTTTGTTGAGAAACGAGATGATTGAG  
 AGGCTATTGTTATCTCACCGTTTGTGAATAAAAAGAAGAAGAAGACAAAAAA  
 AAAAAAAAAAAAAAAAAAAAAAA

10 LJS193 (SEQ ID NO:6)  
 TACTTCGTACTCTCAGAATTCTTACAAGTTCTTTCTCTTAACCTTAAAGTTTATT  
 AACAAAATTGCTCCATTTCGTTCTGAATATTCTGTTGAAATTGATTAATCTATT  
 TTATGTGCAGTTTACTAAAATCCCTATCAGCAACCCGGTGTCTACAGTTGTCAC  
 15 GCTCAGTAGCATCTCAAGGTGGAAGAAAAATGAAACTCCTGCAAATCATCTCTC  
 TCTTCCTGGCTTTCCCGACCTCAAATGGGCCGTGACCGGAATGAAAGTGCAGCAA  
 ATGCAGCTCCCTGCCTGTCGCTGTGGCACGGATGGCGATTCTGCTGCTTCCCT  
 CAGTTGGGAAGCATAAAAAATTAAATTGAACAAACAAATTCTGGATTGATGTTA  
 GCTGAAAATTGAAAGTCTCATTGAGGACTATGAAAGTGGATTGTTATCCAG  
 20 ACAAGCAAATTCAAGGAAGTTGTGAGTCACTTCAGAACGATCTAACACTCGCAAATGGA  
 TTCAATGCAATTGGATTTCTCAGGGTAGTCAGTCCTGCGAGGTCTGTGCAACGATGT  
 TCTTCTATACAAGTAAGGAATCTCATTCCATTGGAGGACAGCATCAAGGGTTTGTT  
 CTGCCCTATTGTCCTCGTTGAGCAGAAAGACTTGCATCAAGCTCCTGAAT  
 TATGCAGCTTATGAAAATGGTACAGAAACTCCTAGTTCAAGCCACCTACTGGCATGA  
 25 TCCTCTAAATGAGGATGCATATCGGACTGGAAGCATTCTGCTGATATAAATAATGA  
 GAGACAAATCAATAATGACTATATTAAATATTGGAAGCTAAATCGTTGTGATGGT  
 AAAGTTCTCAACGACAGCATGGTCAGCCATTGAATCTAGTTCTTGGATTCTACGC  
 TCCAGGAACGTACAGAAGTTCTCCATTAAAACAAAGCAAGATTATTGGAAGATC  
 GTTGGACTCAATCAGTACCGATAGATTCTAGAATGCGGAGGAGATCTTGC  
 30 TTTACAAAAGAATGGTCATAAAGTTATCATACCTATCTGAAGCAATAAGAGCTGCA  
 ATGTAATTGATTAAAAATGTTAACCATTCAGGATGATTGGGTGACCCCTAAATAT  
 AAATGAAAAAATACAAAAGAAATAATTGATCCCACAAAAAA  
 AAAAAAAAAAAAAAA

35 LJS201 (SEQ ID NO: 8)  
 GGATCGGCCATTATGGCGGGGCAGTTAACGCCACAATTAAATAAAATGAGGAACCTT  
 GCTGTAGTCAGTTAGCCGTGCTGTCCTGCTTGTGATGGCCTATAAATGCGGAA  
 GATAATGAAGAAGTTGGAAAGGCAGAGAAAAAGAGGCTAAAGACGCAATGGAA  
 CACTTCAAAAATGGATTAAAGGAGCTGACAAAGGACTTAAACTCCAAGCCTCCAAG

TCTTCCTGGATTGGTAAAAAGCCTGAATCTGGAAGTTCTGAAGATTCTGGAGATAAAA  
CTGAGGATACCACTGGATCTAAGGACGACCAATCAAAGGATAATACGGTCGAAGAATCT  
TAAGAAAGGCGCAAATAGCTATTTCAAAGTGGCGAATGTTCTTCTTATCTGAAATA  
AATATTITAAACCTTCGAAACCAAAAAAAAAAAAAAAAAAAAAAA

5

LJL13 (SEQ ID NO:10)

ACTTAAAGATTGGTAAAGCAAAATGAACCTCTGTTGAAAATTCTCTTGCTCTGT  
CTCTGTGGACTGGGTATTGTCAGGCAGGATGTGAGAAATGCCATCAAACCCCTGGGC  
GTATAGATCGTCCAAAAGAATCCTGAAGATAAGGATCACGTACCTCAATGGAGGAAGT  
10 TCGAATTACCCGACGATGAAAAGACTCATTGCTACGTCAAGTGCATGGACCGCTTG  
GGAGCTTACAATGAAAATGAAAATGTTTCAAATTGATGTCATTACTAAGCAATTAAAT  
GAACGTGGCCTAGAAGTCCGGCTGGACTTGATCAAGAATTGGTGGTCTACAGATGG  
AACTTGCAAAGCAGTTACGATAAATCCATGAAGTTCTCAAATCTCATTATGGACTT  
TAGGAATGCTTACTACGCAACTTATGACGGTCTGATGAATGGTTAGCAAGAACCCCTG  
15 ATGTAAAACCGAAAGGAACAAAAGTTCCAATACTGCAAAAATAAGATGATGGAGA  
TTGCAAACATTCCCTGCAGTATGACTACTACCGCTTAATCGATGAAGACAACTTAGITAT  
TCCGTTCACTACCTGACTATCCGAAGATAAGCTCGAGGAATGCAGGAATGAAG  
CCAAGTCCGCAAATGAGTCAAATCATCTGTTCTATCAGTGGTGGAAATGCGGAT  
AAGTCAGCTTAGACCGCTTGAATATACTCGATGAGTTCTGGAAGATATTAAAC  
20 AAACGGATAAAAAACTTAGGCCAACCTATGATTGAACTTACGATTGAACTTGAAA  
TTCATGTGCTTAACTATTGCCCCTAGGAAGAAAAATCCATATTGGTGTGTTAAA  
CTATTITGAACCTCTCAAAATAACAATTTCAAAAA  
AAAAAA

25

LJL23 (SEQ ID NO:12)

AAAGAGAAGTAGTGAGAATGTTCTTAAGTGGTTGTTGTGCTTGCAGTGTCTTCC  
TTGTTGGGTGAGTCAGGCAGCCCCACCGGGGTTGAATGGTATCAGTGGTCTGATTG  
CTGATATGGACAAAAATCCATCGCAGTGACAAACCCACCTTAAACAGCGTCTAAAG  
30 ATCGATGAATTGCCACAACACAAAAACGGATCAATACATTATGCGTAGTCGAGT  
GAAGAAGCCCCTTCCACGAGGTATGGTTCAAAGGACCGGGTGCAGGAAATTGTCGGAA  
TTGTTGTCTCAACAATAAAACTTACACAGTTGATGATAAATCTGGAATTACGTTCCGCA  
TAACGAAAGACGGAAAATCTTCCGTGGTTATTCTCGCAGATGCCATGGACAGCGA  
CCCGATGGCTTAAGGGTGAATGGCTACAATTAGGATGATACAATCTATGTTGGATC  
35 TACGGGGATGCTCAAGTCACCTCATCCCTTGGGTGAAGAAGATCACGAAAGATGGCG  
TTGTTACGAGTCACGATTGGACTGATAAATACGAAAGATTCTCAAAGCTCTAAACATG  
CCAAATGGTTGTCTGGCATGAGGCTGTTACGTTCTCCATTAGGAAGCAATGGTC  
TTCATGCCGAGAAAGTGTCAAGGCATCCCTCTCACAGGAACCTCGAAGAACGCACAGG  
GTGCAATAAAATAGTGACGGCAGATGAGAATTCAACGACATTCAAGTTATTCACATT

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AAGATCAGCCATATAATTAAGCTCTGGTTCTTCCTCCGCTTATTCCCTGGTACGAA  
AAATGAAAGACTTCTGCCTTGAGGACAGTAGAGCAGGAAGATCAGGTTAAAACCTGGG  
CTGTGGTCATGGATATGAAAGGAACAGITCTGATGTACGAAAAGGAACCTTATGACGAA  
AAATTGAAAGGTTAGCATTCTGGGTATTAAAAAGAATTAATTGTTCCAGAAGCT  
5 TTTAGATGAAATAATAAATTTATTCATTAAAAA  
AA

LJM10 (SEQ ID NO: 14)

CGCGGCCGCGTCGACCGACAGAAGGGTAGTTGTAGAGAACTTGAGTTCTAAAGGAA  
10 ATTCTCAAGAAGAAAATATTCAAAAGTAAAGAATGGCGTTGAAGTTCTCCCGGTTCTCC  
TTCTAAGCTGCTTCGCAATGAGCACGGCACTACAAGTTACTGAGAAGGAACCTTCTGAT  
GGGAAAAAGATCTCATCTCAAAGTTGAGCTAAACTGGTCGAAGCTCTGATTCTGT  
ATCCATCGTGGTCTTACGTTGCTCTCAATTAAATCCGCCAAGGAAATGTAGACGTAACA  
AAAGCAATTGGGCTGAATTGAATTGATTCAAAGAAATTGGCTATGTGTGGACTGG  
15 AGGTATTGCCATAGTCAAGATAAGTATTCCGGATAATGATGGAACCTAAAGTTG  
TTAACGAGTCTACACCAATTGGTCACTGGAGAACCAAATAATGGTACTGGAAGGAT  
GAATTGGCTGGAAATTACTATAAAACGAAGAAGGGAAAGTGGATGATGATAATG  
TCACGTGAAGCATCATTGTATGTCAAGAAAAGAAATAATTGATTGATTGTTGCT  
GATTGCAAGTCAGAATTGAAAAGCCAAAA  
20

LJL143 (SEQ ID NO: 16)

CTCTTGGAATTATTGAGTGATTAACAGGAAATTAGCTGAAGAAATGAATTGATTAAT  
TTCCTATCAATAGTTGGTTAACAGTTGGATTCTTGCAGTAAAGTGTGATGGT  
GATGAATATTCAATTGGAAAATACAAAGAAAAGATGAGACACTGTTGGCAAGCTA  
25 CGGCCTAAAGAGGGATCCTGCCAATTGTCTTAGGCTACAAATGCTCAAACAATCAA  
CCCACTTGTGCTTAATTAAACCAATAAGAAATCCTGCATATCAGCAATTAGCTGA  
CTTCTTACCCAAAATCAATCAAAACTCGGATTAACTAAAAATCTCTACTGCCAAACTG  
GAGGAATAGGAACAGATAACTGCAAACCTGTCTCAAGAAACGTAAAAGACAAATAGC  
AGCTAATATTGAAATCTACGGCATTCCAGCGAAGAAATGTCCTCAAGGATCGTTACAT  
30 TGGAGCTGATCCACTCCACGTCGATTCTATGGGCTTCCGTATCAGTTGATCAGGAACA  
TGGATGGAATGTGGAACGATATAACATTCAAAGACACAAGATTTCACAGAAGTTT  
TCTACCACAAAATGGTTATTAAACACCCAAATAACTTATTGGCTGAAGAAGATTCC  
TCTCTGAAGCTCGAGAGATTACTGCGAAGGATATTAAGAAGAAGTTCAATTATTG  
CCAATGAAGAGTATAAGAGGATTAGTTCTGGACGTTATTGGTCCAGGAGACTATGC  
35 GAAAAAAGCTAAATATCCCTACATTCACTACAATGGAGAATGCAGCAATGAGAATAAA  
ACTTGTGAACCTGTCTTGACACCGATGAACTAATGACCTACGCCCTGTTAAAGTCTT  
ACTAATCCTGAGAGTGTGATGGATCTAGGCTCAAAGAAGAGGATTGGAAAGAGGATAAA  
TCTTCTTAATAAAAAAGTTCTGTAAGAAAATATTGTTCAATAAATTAAAA  
AAAAAA

LJS142 (SEQ ID NO: 18)

AATAGATCTCAAAACGTCTAAGAATGGCTTCAGCAACACTTATTGTTCTTTGTG  
AGTTTTTAACGTTTGTGGCGCTGATCAGACACTTATTGAGAAGGAATTAAACCGGAAGA  
5 ACTGTTATATCTCAAAATTAAAGCTAAATTGGAACGATGCCTCGATTACTGCATCCGC  
AATGGCCTCACCTTGCTAAGATTAACTAGCTGAAGAAAACACCGAACTGAGTGAGAA  
ACTCAAGACAGTCATCGTACGGAGGAGTTCAAGTTGGATTGGAGGCATTGAACATC  
ATCAAGACAGTCATCGTACGGAGGAGTTCAAGTTGGATTGGAGGCATTGAACATC  
TACAAATACACAAACTGGAATACCGGAGAGCCCACAAATTACCAAAACAACGAATATT  
10 GCTTGGAAATATTATTCCGGAAGGAAGATGGAAATGGAATGATTTCCTGCAGTGCA  
AGACATCATTGTTGTGAAAAAGAACAAAATAAAATGAAGAAAATGTGATTTCCT  
TTGGTTGAAGAATAAAATTCTGTTGAAAAAAAAAAAAAAAAAAAAAA

LJL17 (SEQ ID NO: 20)

15 ATTTAGTTGTGTTAACAAAACAAGAATGCAGAACTTCCTTTAGTTTCCTGGCTTAG  
CTGCCTTAATGCTATGTGCCGAAGCAAAGCCGTACGATTTCCGTTATCAGGACTAA  
TTCAGGGCGTTATTCAGCGCGAAAGTCAGCTGAGAGGGAGAAGAGAAGGCCAATGA  
GGACTATGAGAAGCAATTGGGGATTGTTGATCAAATTAGGAAATTAGTTCAATG  
TCATGAAAATGCCCATTTGGAAGCTGATGATAATCGTGTGAGTGGCGAGTACGTTG  
20 ATCATCATTATGGTACGAAGATGATCGTGTGATTATGATCATTACTAAACTACTTGCTC  
CTGCTGAATGACTGAAGGAATCATTTTGCAAAAATATCCATCAAATTATTGAATTA  
ATAAAGTTGCAAAAAAAAAAAAAAA

LJM06 (SEQ ID NO: 22)

25 GTTTAAGGAATTCTTCATCTCAGTCTCGATTTCTTAAACAAATAATGAAGTTTAT  
ATTTTGGAGTTCTGGTGAGCTTCTGCATTATGCAATGCTGAGGATTATGATAAA  
GTAAAACCTACTGGAAGAACTGTTACATCTCAGATCAAAGGCTCCGTGGTCACAGCT  
TTAGACAATTGTAATCGTTACGCTTCACCTCGCCATGATCAAGTCTCAGAAGGAGAAT  
GAAGAGCTAACAAATGCGCTTAAAGTGTAAATTAAATCTGACGAAGAAAATGTTGGAT  
30 TGGAGGTCTAGGCACGATCTGGATGACTACTCCGTTGGATTAGTTGGAAC TGCAATT  
GTCAAAGACTCGTACACCAATTGGGCCAAAGGAACCC  
ACAGGAAGGCCCATAGAACTCAAATGATGAATTCTGCATGCAAATGTCTTCAAAGA  
TGGTGGCAAATGGAGTGATAACACCTGTTGGCGTAAACGTTGTACGTTGTGAAAAGC  
GTGATTAATAAGAACACTGCCAATGAATATTGGCAATTGAGAGAAATTAAATTA  
35 AAAAAAA

LJM17 (SEQ ID NO: 24)

AGTCAGTGTAAATGAAGAAATTGCAATTATGAGGTTCTCTTGTGTTCTGCCATCGTC  
CTTTCAAGGGATCCACGGAGCTATGTGGAAATAGGATATTCTCTGAGAAATATTACA

-30-

TTGATGGATTGGATACAGATGACTACAATCCAAAGTTAACATTCAACGGGTTGGC  
 AGTTGATCCCGAAGGATATAGGCTCTCATGCCATCCAAAGGAGAAAGCCAAAGGTT  
 CCTACACTGTGGCTGAAGTGAATATGGTCATGAATCCGGATTCCCGTCAGAGAGCTC  
 CGAGCTTGAGAAATTCAATGGCGAGGGAAAAAGGATCTTGTAAATGTG  
 5 TATCAGCCAGTCATTGATGATTGTCGTCGCTTGGGTGCTGACATTGGAAAGGTGGAA  
 TACACCGTGGTGATGCTGATCAATATCCAAAGGAAAGCCTACCTAATTGCCTACGA  
 CCTCAAGAAGGATCATACTCCGGAAATTGATCGATTGAAATTCCAGACGATCTCTATAG  
 CTCACAAGTTGAATTGGTGGATTGCCGTTGATGTTGITAACACGAAAGGAGACTGTAC  
 GGAGTCATTGTCTACCTGACCAATTCAAGGATAACTCTCAATTGTCTACGATGAGAC  
 10 ACAAAAGAAAGCTGGAAATTACAGATAAAACATTGAAGCTGATAAGGAATCCACGT  
 TCTCCTACTCGGGAGAGGAACAAATGAAGTACAAAGTCGGCTTTGGATAGCTCTG  
 GGTGATAGGGATGAAATGGGCATCGCCTGCCTGCTACATCGCTGGAGTAGCACCAA  
 AGTCTACAGTGTAAACACTAAAGAACTCAAAACAGAGAATGGTCAGTTAAATCCTCAGC  
 TTCACGGTATCGTGGAAAGTACACAGATGCAATTGCCCTAGCCTACGATCCTGAGCAT  
 15 AAAGTCCTCTACTTGCTGAATCCGACAGCAGGCAGGTGTCCTGTTGGAATGTAAATATG  
 GAGCTAAAACCAGACAATACGGATGTGATCTCTCTAGTGCCTGTTACTTTGGAACG  
 GATATTGGTTGATAGCAAGGAAATGCTGTGGATAATGGCTAATGGACATCCACCAGT  
 AGAGGATCAAGAGAAGATTGGAAGATGAGATTGTAACCGGAAGATCCGTATTATG  
 AAAGTGGATACGGAACGTGTTCAAATATTACGCTGCAATCAAATTATAAGCCCC  
 20 AAAGGAAATTGAAGTTGAGACACAGGAAAAGCTCAATTCAACAAGAATTGATCT  
 TAATCTGAATACCTAAAGTCTGTCAAAGAATTGATATTATTGAAAACCAATAATTG  
 ATTAATTTCGAAAAAAAAAAAAAAAAAAAAAA

LJL04 (SEQ ID NO: 26)

25 ACTAAAGCGTCTACCGAAATCAGGGAAAATGATTAAGGAAGTTCTCTGGCTCTA  
 CTGTCGGCCTGGCACAGTGTGCTAATGAAATCCCTATTAAATCGTCAGGGAAAGATTAT  
 CCAGTTCCGATCATTGATCCAAATAATCATCTCGGATGATTATTCGATGATCGCTTC  
 TACCCCTGATATTGATGATGAGGGCATAGCTGAGGCTCTAAGGATAATAGGGAAAATC  
 CCGTGGTGGTGGTGGCGCTGGCGCAAGAGAAGGTAGGTAGGTACGAATGGGGCTAAA  
 30 CCGGGTCAGGGTGGAACTAGACCAGGACAGGGTGGAACTAGGCCAGGACAGGGTGGAA  
 CTAGGCCAGGTGAGGGTGGAACTAGGCCAGGTGAGGGTGGAACTAGACCTGGCAAGG  
 TAGAACTAACGCTGCTCAGGGAAACTACTAGGCCAGCTCAGGGAACTAGAAATCCAGGAT  
 CGGTTGGTACGAAAGAACCCAGGATGCGTCAAACAAAGGTCAAGGTAAAAGAAGGCC  
 AGGGCAAGTTGGTGGTAAAGACCAGGACAAGCAAATGCTCTAATGCAGGCACTAGA  
 35 AAGCAACAGAAAGGCAGTAGAGGCCGTTGGAAGGCCTGATCTATCGCGCTACAAAGATG  
 CCCCTGCTAAATTGTTCAAATCTCCGATTCAGTGGAGAAAGGAAAACCTCAAATG  
 TAAATTACTTTAGAACGAAGAAGAAGGAGCACATTGTGACCCGTGGTAGTCCTAATGAT  
 GAATTGTTCTGGAGATTCTCGATGGGATCCAATGGCCTGGACTAAAGAGTGAAAC  
 CATAGGCAAAGATAACGCGTTAGTGCTGGAGAACCTTAATGGAAATTCCATCGTGGCTC

GTGTTAAGATCTACAAGAACGGTTATTCAAGGATGAAGAAGAAATCCTTGATTCCCCCC  
CCCCCTTCCTTAAAATTCAACATAATAAAAAAAAAAAAAAA

LJM114 (SEQ ID NO: 28)

5 GTCTTTCTGAGTGTTCATTAACAAAATGAATTCAAGTAAACACTTAATTAACTCTT  
CTATTGCAATTTCATTAGTAAAAGGTCTCAGGCTTCTTCATCTGACCCAAGTA  
TCTGTGTTAAAATTAGTATTGGATACAGGAAGGACTTGTGAGGAAAGTGAATATTTC  
CGGATATCAAGAACGTTAAAATGGAAAAGAGTTACATTGTCTGCACTGATTCAAGAT  
GCAGTTGATTATAAATTATTTGTTGATATGAATCGTCTTCTGGACCACCGTATC  
10 CTGAGGAAGAAATCCTCGTGAATCAACGTAACCTATGCCAAATTATGAGCTGATG  
ACTACGGAAACCACTGAAACCAAAAAGCCAAAAAGAAACCAAAGAATTCAAAACGG  
ACCCAGACCCCTCCAGCAATTGTCCAGGATTTCATTTAGAAATTCAATTCTGTTAATT  
TTACAATTATTGAAAGAAAATGATATTGAAATATTCTATACAAAAAAACAACA  
GTTATAAAACGAAAATTCAATCATTCATGAGAAAACCTAGTCTGAGTAAGGTTATT  
15 CACCACCCGACGCCACGCTATGGTGAATAATTCTTATTCACCACATCAAATGACGG  
CTTATAAACTTCACAAATAGTTGGAAAATACATTCTAACTATGCAATGTTACTTA  
AAATCACTTACAAATTCACGCATTGAGATGCAACAAATATACAATTCAACGATAT  
AAACTTCCACAAGGAAAATTCAACCAAAAAAAAAAAAAAA

20 LJM111 (SEQ ID NO: 30)

ATCATTCAAAAGGCAGCAGCACAAATGAAGTTATTCTTCTTACACTTTGGTCTAGT  
CCAAACGATTTGGAGTAGAAATTAAACAAGGATTAAATGGAATAAAATCCTTATG  
AGGGCGATACATCAGAAAACCTCAATCCAGATAACAAACATCCTACGGCTTGTAC  
GATCCTGAGAGTCAGAAACTCTCTTAACGTCCGAGGAAATATCCGAAACTATGTA  
25 CACTTGGCAGAAGTTGATACTGAGAAAATTCTTGAATCGGGAGATACTCCCCGCT  
CCTTGGAAAATTCACTGGTCTGAAACTGGGAAAGAACTACATCAGTTATCAGCCAG  
TTATCGATGAATGTCATCGTCTTGGGTTGTGATGTTGGATCAGTAGAACGTAACCTCAG  
ACGGCACAGAAGGTAGCCAGAACATAATCCTACCCCTGTGGCGTACGATCTCAAAGAA  
GCCAACTATCCTGAAGTTATTGTTACAGCTTCCGATAATTCCATTGAGAACGCCACA  
30 TTTCTGGGTGGATTGCCGTTGATGTTGAAAGCCGGATGAATGCAGTGAAACTTTGTC  
TACATCACAAACTTCCTCACCAACGCCCTCATAGTATACGATCATAAGAATAAGGACTC  
CTGGACGGTACAAGATTCAACTTGGACCAGATAAAAAGTCAGTTGACCACGATG  
GACAACAGTATGAATACGAAGCAGGAATCTCGGGATTACCCCTGGAGAGAGAGATAA  
CGAAGGAAATCGTCAAGCGTACTATTAGTAGCAAGTAGTACCAAACATTCAACAGCATCA  
35 ACACCAAAGAACTGAAGCAAAAGGAAGCAAAGTTAATGCAAATTATTGGGAGATCG  
TGGTGAATCCACCGATGCCATAGGCTTAGTTACGATCCAAAACCAAAACTATCTTCTT  
CGTTGAGTCAAATAGCAAAAGAGTATCATGCTGGAATACCCAGGAAACACTAAACAAG  
GATAAAATTGATGTAATCTATCACAAATGCAGACTTTCTTGGAACAGATATATCGATT  
GATAGTCAGGATAATTGTGGTCTAGCAAATGGACTTCCACCTCTGGAAAATTCTGAT

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AAATTGTCTTACAAAGCCACGTTATCAAATTCAAAGTCACATTCAAGAAGCAATT  
GCTGGAACATAATGTGAAAAGAACCTTAACAAATGAAACTTGTAGAAAAATACATAA  
TATCTGAATAAAAAGTCATAATGTACCATAAAAAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAA

5

LJM78 (SEQ ID NO: 32)

CTTAAAGCAAAATTTGTGGAAAGGAAGTTACCCGGAGATGACGTTCTAATTATA  
CTTGGTGCATTCTCCTGTTCAAATTATTACAGCTTCAGCTTAGGATTGCCTGAACAGT  
TTAAAGGTTAGAGGATTACCTAAAAACCTTGGCAGAGACTTATTATCACGAAGGA  
10 TTGAATGATGGAAAACGGATGAAATGGTGGATATTAAAGTCTTAGCGATGAATT  
TAAATTCACTGATGAAAATTAGATGTTGGTGGAGGAGAAAAATTACAAGAACGTGATA  
TAACCCAAAATTCACTGGCAAGGAACCTCCTATCAAACGTAAGGGATTCCCTCAATG  
CCATCACTCCCTCAATGCCCTCAATGCCATCAATTCCCTACTTGGTCAAGTCAGACA  
CAGGCAGGACCAAATACCGCACTTGCCTCCTGAATCTGATTATTCCCTCTAGATATG  
15 CCGAATATTGTGAAAATTCTAAAGGAAACAAGAGACCTCTATAACGATGTTGGAGC  
TTTCTTAAGGCAATTACAGAAGCTTAAACAATAGATCTCATCTCAACTTCTTCC  
TCCCCAATGGTGGAGCACGAATAAAACCAAGAACATTATTCTGGAAATGAAATACAAAAGT  
CCGAAAAGTGAGAAATTCTGCCAGGAAACTCTCAGAAAATCCGAGACATTCTGCTG  
CTATTGCCAAAAGGTAAAATCATCAGAATGTCTGCAATCTTACGGACATCAAAGGA  
20 CTTGTATCAGACGGAATTATGTTAAAGGAAAATTCAATGATGGAAAACGAATTAT  
CCTGCAATTGTACAATAATTACTAAAAGGACTCAAATTCACGCTAATGGTTG  
AATTGAAGAAATGTGATACAAATCAAACAAATCTTGGGAAGAATAATCTGTTATT  
TTGACACCATTGCACTGGAAAAAGAACAAATTCTTCTACCTGTAGAATTATAAGCG  
CATTCTGAATTACCCACTACTTCCACAATGAAAGAACATCTTACGGACATCAAAGGA  
25 CACAACGATTGCATCCATTACGTAAAAATGGAAAATGTGCCGGTAAATGCTTGAAA  
TCACCAAAGAAATTCTACGCAAATAACAGTCCAGAATAACCAAATTAAATGATTACT  
TCTCAAGGAAAATACTACCAAAAGGCATTAATTAAAACGATGTTTTATAAACAAATGT  
AAGAAAAAAAAAAAAAAA

30 LJS238 (SEQ ID NO: 34)

AGTTAATCTCTGTCAAGCTACAAAATGCTAAAATCGTTTATTCTATCAGTTTGGC  
TGTATTAGTGTAGTTGTAGCAGCAATGCCAGGATCCAATGTTCTGGCACATTCA  
AGAAGAGCTTGAGAAGCTCGTGAAGCTCGAAAGAACATCACAGGCACTCGAGAAGGCA  
ATTGATGAATTACGAAATATCTCTGATTGAAAGAGCAAGGAAGAGGAAATAAC  
35 GGCGAGGAAGGATTCTTAGAGATTCTCTTTATTACTCAAACCTAACCTCAA  
ATCAGTCTGATATTCTTAATTGAAAAAAATTGAAAATTAACTATTGTGAAATT  
TAAATAAATAAGAATGTCAGAAGCAAAAAAAAAAAAAAAA

LJS169 (SEQ ID NO: 36)

AATTTCACCATGAAGTTTCTGCCAGTTCTGCAATTTCCTTGCGGATT  
ATCGTGTGAGGGTCATCACAATGTGAAGAAGATTAAAAGAAGAAGCTGAAGCTTC  
TTAAGGATTGCAATGAAGCAAAAGCCAATCCTGGTAATCAGAGAAATCTCACCAAAGA  
5 AGAAATGTTGAAGAATTGAAAGAATATGGAGTTGCTGACACAGACATGGAGACAGTT  
ACAAACTTGTGGAAGAATGTTGGAATGAATTAAACAACAAACGGATTGTAAGAGATTCTC  
GAAGAGGCTGAATGCTCAAGAAGAAGAATATTGTAATTTCCAGATGAAGTGAA  
ATTGAAGAAGAAATAAATTAGCTGAAAAAAAAAAAAAAAAAAAAAA  
10 AAAAAAAAAAAAAAA

10

LJL11 (SEQ ID NO: 38)

AGTTGCAAGAATTCTCATTGCGTAAGATGTTGTTCTTAACCTTTGTGCTGGT  
GTTCATAGAACTGGCGTTGTTAACAGCATCAGCAGCAGCAGAAGACGGCAGCTATG  
AGATCATAATTCTCACCCAATGATATGCACGCGCTTGTCAAACCAATGCTGGAA  
15 GCAACAAATGCCAAGAAAAAGACAAGATTGCTTCAAATGCTACGGAGGATTGCAAG  
AGTTCAACAATGGTAAAAATTCCGAGAAGAAAATGGCAGCAGTGTCTGTTCTGA  
ATGCTGGTACACGTATACAGGTACCCATGGTTACCCCTACAAGGAGACCATTGCA  
ACGGAGATGATGAACATCCTCGTCCAGATGCAGCCTCACTGGAAATCATGAATTGCA  
CAAAGGAGTAGAAGGACTCGTGCCATTCTCAATGGTGTACCTCCATTAAACAGC  
20 GAATTGGACACTTCTCAAGAGCCAACAATGACCAATGCTAAAAATCTCAAACGCTCAA  
TGATTTTACGGTTCCGGGACAGAGTTGGTGTAAATTGGCTACCTAACGCCTGATA  
AATTCCCTCGGACGTTGGTAAAGTTAATTATTCCGGAAGTTGAAGCCATCAATACGG  
AACGACAGCGTCTGAAGAAAGAGGAAAATGCCAAATAATCATCGTTGGACATTCA  
GGGTGATAAAAGATCGAGAAATTGCAGAGAAATGCCACTGGTGTACATAATTGTTGG  
25 AGGACATTCACACACATTCTACACAGGAAGTCAGCCTGATCGTGAGGTTCTGTAG  
ACGTTTATCCTGTTGACCCAAATCCAGTGGGAAGAAAGTTCAAATTGTTCAAGCCT  
ATTGCTTACAAAGTATTGGGGTACTTAAAGTGACGATCAACGGAAAAGGAAATGTT  
GTGGGATGGACTGGCAGCCAATTCTCTTAATAACAAACATTCCCCAAGATCAGGAAGT  
TCTCACTGCTCTGAAAAGTACAGAGAACCGTGGAAGAAACTATGGAAATCGCGTAATTG  
30 GAGTTCCCGTGTAAATTCTCAATGGGGGCATACTGAATGCGTTCCATGAATGCAATA  
TGGTAATCTCATCGGACGCTTTGTTGATGCCAATGTAATCAGTACACCAATGAGTA  
CGAATGCCTGGACAGATGCAAGTGTGTTCTGTATCAAAGTGGTGGCATTGTGCCCCA  
ATTGATCCTCGTACCGCGGCAGGGAGCATCACACGCCTCGAGTTGGACAATGTTCTACC  
ATTGGGAATGCACTGTACGTCGTAAGGTTCTGGGAATGTCTTACGCAAAGCTTGG  
35 ACATTCACTGATACTCCAACACTTCGGGATGGGAGAATTCCACAAGTTGG  
GCTAAAGATTGTTAACGTCAATGAAGAAATTGGAAAACCGTAAAGTCCGTTAAAG  
TTCTCTGTAGCAATTGCTCTAACCTGAATACCAACCAACTGAGAAATAAAACTTAC  
AACGTTATCATGGACAGTTTATGAAGGATGGAGGTGATGGGTATAGCATGTTCAAGCC

CTTGAAGATCATCAAGACCCCTCCACTGGGAGATATTGAAACAGTAGAAGCTTATATTG  
AGAAAATGGGCCCATTT  
TCCCAGCAGTCGAGGGAAGGATCACTGTTCTGGGGACTTCAAAATCAGATGAGGAT  
TGGCATTAGAAACATCCTGGACGTTATGGAAAGAATAAAGAAGGATCATAGAAAAAA  
5 AAAAAAAAAAAAAATAAAAAAAAAAAAAAAAAAAAAAA

LJL08 (SEQ ID NO: 40)

GTCAGTGATCTGATAAGTTATTAAAATGAAGCAAATCCTCTAATCTCTTGGTGGTGAT  
TCTTGCCTGCTTGCCTCAATGTTGCTGAGGGCTGTGATGCAACATGCCAATTTCGCAA  
10 AGCCATAGAAGACTGCAAGAAGAAGCGGATAATAGCGATGTTGCAGACTCTGTAC  
AAACAACAGCAACATTCACATCAATGGATACATCCCAACTACCTGGAAATAATGTCITC  
AAAGCATGCATGAAGGAGAAGGCTAAGGAATTAGGGCAGGAAAGTAAGAGATTGAGG  
AAAATTGTAGCCGAAGAGAGAAGGAAGGAAAGTCCCATTTCGTTGTTAATTGTAAC  
GAATTTCGCAAAAAAAATAAAATATTATGCACTCCAAAAA

15 AAAAAA

LJS105 (SEQ ID NO: 42)

TATTTTAATAATTCTGTGTAAAATGAACGTTCTTCGTGTTACGCTCACAAATTCT  
TCTTCTCTGTGTTAGGCACGCCAGAAGATTCTGTAGCTTCAGGATCAAGCTAATT  
20 CCAGAAAATGCCTCGAACAAATATCCAGAACCAATCAATCTGGAGAAGTTCTGCGTGCC  
TCAAGAAGCGCGAAGGTGCCAAAGATTCCGGAAAAGAGGGAGCCTGGATGACATAGA  
AGGGACTTCCAAGAGTCTGGAAATCTCTGGGTGCATAGGAAGCTCAGAGGACTTCTA  
ATCAATCTGTGAGAAGAGAACCCACGGCTAGAGAAAATTAAAGGAAATAAGAAAT  
TAATGAAGCATTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

25 AAAAAAAAAAAAAAA

LJL09 (SEQ ID NO: 44)

GTATATCAAGTATCATTCAAGTGAATCATTGGCTCCGTAATTGTACAAAGAAAAAA  
AAGTTGATAAAATCATGAAAATCACTGTGATTTATTACCGGGATTACAATTGCCCTCG  
30 TGAGTAGTGCTGTGCTTAAGAAAAACGGTGAACATTGAAGAAGAAGAAGTAAGAGC  
TGAGCAACGACTTAGAGAGATCAATGAGGAACCTGATCGTAGGAAGAATATCAATACTG  
TAGCCGCTTGGCTTATGCATCCAATTACTGAGGTCAATCTCAAGAACATGAATGATG  
TGTGGTTGAAACCGCGAAATACTACAAGGAACCTGCATCTGAATTGAAGGGATTCAAT  
GCCAAGGAATACAAGAGTGAGGATCTGAAGAGACAAATTAAAGAAGCTAAGCAAGTGG  
35 GATATAGTGCTTACCATCTGAGAAGTATAAGGAGCTTGGAGCTATCACATGGATG  
GAATCGAATTATGCAAAAGTGAAGAGTTGCTCATACAAGGATCCAAGAAATGTGATT  
AGCACTTGAACCTGAAATTACGGAAATCCTTATTAAAAGTCGAGATCCTGAGGAACCTA  
AATATTATTGGAAACAATGGTACGACAAAGCTGGCACACCAACTCGAGAGAGTTAAT  
AAGTATGTACAACAAATCGTGAAGCAGCGAAATTGGATGGATTTCATTGGGTGCAGA

ATCTTGGCTTGTGAATATGAAGATGAGACATTGAGAAACAACCTGAGGATATCTCG  
 CCCAAATTGCCACTGTACGAGCAACTCCATGCTTATGTTAGATTCAAGCTGAGGGAA  
 AAGTATGGAAATGACGTTGTCGGAGAAAGGTCCATTCCAATGCATCTCTGGGAA  
 CATGTGGGTCAAACGTGGAGTGAAGTTGCCAATTAGTCCATACCCGAAAAGA  
 5 AGCTCCTCGATGTTACCGATGAGATGGTAAGCAGGGATAACACACCAATTCTATGTTG  
 AAAAAGGAGACGAATTTCAGCTGAATATGACGAAACTTCAAAAAACCTCTGG  
 GAGTACAGTATTTGGAAAAACCCAAAGATGGTAGGAAATTGATCTGCCATGCAAGTGC  
 ATGGGACTTCTATACAAAGGATGATGTAAGGATTAAACAGTGTACCAAGAGTTACAATGG  
 ATCAATTCTCACGGCTCATCATGAGCTGGTCACATTCAATATTATTGCAATATCAAC  
 10 ATTTGCCAGTGTACAGAGAAGGTCCAATCCAGGCTTCACGAGGCTGTTGGGAT  
 GTTCTCTCTTCGGTATCAAGTCTAAACATTGGAAAAAGTTGTTGCTAAAGAC  
 TTCAAATTGATGAAGAATCCCAGATAAAATCAACTTCTAAATTAGCTCTGGATAAAATG  
 GCATTCCCTCCATTGCCTATACCATTGATAAAATATCGCTGGGTGTGTTGGGTGAA  
 ATTCGCCGTCTGAGTACAATTGCAAATTGGAAATGCGTCTACTATGGTGGTATA  
 15 GAACCACCAATTGACGTTGAGAGTGATTTGATCCACCAGCAAAATATCATATTCA  
 TCGGATGTTGAGTACCTCAGGTATTGGTTCTCATTATTCACTGTTCCAATTCCATCAAG  
 CTGTGTGCCAAAAGACTGGTCAGTCGTAACGAAATGATCCGGAGAAGACTCTCTAAAT  
 TGTGACATCTACCAAGAGTGCTGAGGCTGGTAATGCCTCAAAGAAATGCTCAAATTGG  
 ATCCTCAAAACCATGGCCAGATGCAATGAAATTCTACGGGGCAAAGGAAATGGATG  
 20 CTTCTGCATTAATTGAGTACTCCGTCACTCAGTGAGTGGTTGCAGAAGAAGAATAAG  
 GAACTAGGAGCTTGTGGCTGGACAAATCTACTAAGTGTGTCAAAACGTCAGTTA  
 ATTTTGAGCCCTAAAAAATTCAACATTCAATATGACAAAATATGATTT  
 CGTAAAACTAAGCATGAGTAAGTTTTGTGAATTAGCAGTTCAATTCAAGAAT  
 AAACGTCAAATTAAAAA  
 25

LJL38 (SEQ ID NO: 46)

TCAGTTAGTTGACTAACAAACCACAATAGAGACACTAAATGAAGACATTGCCCTAAT  
 CTTCTTGGCTTGTGCTTTGTGCTCTGCATTGACGGAGCTCCAATTGTGAATTAA  
 CTGGACGACGTACAGGAAGAGGTAGAAGTTAACAGTATGAGCTTAGGAAGAAAATG  
 30 TTTGAGGAGTTTCAGGCAGAGCAGAGCTTCCCAGAGAGGGAGCTTGCCTGCTGT  
 AGATTTTAAAAATGAATCAATTGATTGGAGCAATTACGCTATATTGTGGAAATT  
 TTGAATTAAAAACTAATTATGAAATTAAATATAATTTCAGAATTCAATAATTCA  
 CAAAATTGTATTAATTAAAAATTGTATGAAATTCCAATAAGCTTCAAATTAAA  
 AAAAAA  
 35

LJM04 (SEQ ID NO: 48)

GGCCATTATGGCCGGGGATAGAACATTAAATTGTTGTTAAATGAATCACTTGTGCTTATT  
 ATTATTGCTCTATTCTTTGGTCAACAATTGGCTGAACATCCAGAAGAAAATGT  
 ATTAGAGAATTGGCGAGAACTGATGAAACTGCATTCTCATTGTACGTATTGTACTAC

GGATTCGTTGATAAAAATTCAGGATCGCTAAAAAACATGTTCAAAAATTCAAAAAAAT  
CCTAGTTACATTGGCGCTGTCCTAAGAAAGAAAAAGAAACTTTAGAGCACATTG  
AGGCTTGTGCGGATTCTGCGAATGCTGATCAACCTCAAACAAAGATGAAAAATGTACA  
AAAATAATAAGTACTATCGTTGTGGATGGAAAAATATTACCTGGAATAGTTA  
5 TGCTGATGCAATCATTAAGTTGATAAAAACCTTAACGTATGAAGCAAAGATATTGAA  
AAAAAAACATCAAGATTATGCTGGAAAGAAAAAATAAAAAAAATTGTGCTAATCAA  
ATTGAATTAACGCTTAATGCTATATTAAAAAAAAAAAAAAAAAAAAAA

LJM26 (SEQ ID NO: 50)

10 GTCGGAGATCGTCTGCCCTGATGATCACATCGTATTGTGAGTTACAAGAGTGAAACTT  
TTAAGTGTGTGTCTAGCAAAGTGATTCCACAATGAAGATTATTTTTAGCCGCTT  
TCTACTAGCGGATGGTATTGGCTGCTGAAGAACCTCAGTGGAAATTGTAACACCAC  
AATCAGTGGAGACACGCTACGCCAAAGCCCAGGACGCGAGGGTAGGAAGTGAATC  
CGCAACAAACAGCACCAAGACCAAGTGAATCAATGGATTACTGGAGAAATGATGATTG  
15 TCCCATTGAGGGCATTCAAGGATATTGGAGAATTGACTGGAACCTTCGAAGATCG  
TTTGAGGAAAACAAAGGTAATGCCATCTGTCGCCACTCTGTGAAGCTACTAATGA  
GTTGCTCTCGAGGCCAGTGCAGGTACCTGACCCAGCACCAACTCAGACAAGCC  
ACTCCCACCATCGTCACCCACTATCAGTCTCGAGAATTACAAGAATATCTTGACGGT  
CTCAAGAAAAGAGTAACGACTACACGGTCACTTGGTACGAGAATCTACGTGGATCA  
20 GTTGACGCCCTGCCAGAGATATGCCATTGGAGAAGCATTCTGACTGATCT  
CAAAGTTGAGGACTCTCGAAGGAAAAGAAACAACTCAGGAATCAATAGTTGGGTGT  
CAAACATCACAAATGAGCACATAAAGGATCTGTGAAGGAGGAAGATGTTAGAATT  
AGTTATGCTCATGCTTAATGCAGTCTACTCCCGGACTCTGGCGCAAGCCTTCATCG  
TACACTCCACTGCCCTCCACGTGAGCGCTGATGAGTCCAAGACGACTGATTTATGCT  
25 AACCGATGGCTCTACTACTTCTACGAGGCAAAGGAATTGGATGCTAAGATCCTCAGAA  
TTCCCTACAAAGGTAACAAATACGCAATGACTGTGATCTTACCAAATTCCAAGAGTGGC  
ATTGATAGCTTGTGCGTCAGATTAACACGGCCTCCTGCACAGGATTAAGTGGTGTGATG  
GATGAAGTGGAGTGCAGGGTTATTCTACCAAGTCCACTTGACATGACGAATGAGCT  
GAAGGAATCGCTCGAAAGTGGCATCAGTCAGATTTCACATCAGAGGCATCTTGC  
30 CATCATTAGCACGAGGACAGGGCGTACAGAACATCGTCTGCAGGTGCTAATGTGATTGAG  
AAGGCAGGAAATAATTGTGGATGAGAACGGCAGCACAGCCTATGCTCGTCAGAAGTGA  
GCCTAGTCAACAAGTTGGAGATGATGAGTCGTATGTTCAACGCTAACATCCATTCC  
TCTTACAATTGAGGACGAAACCAACGGCGCAATCCTATTACGGAAAAGTCGTCGAT  
CCCACGCAATAGGAAATGAAAAGCATTCTACGATACAAACTTTTTTAATTAATTAT  
35 TCCTCATTGAAGGACATTAATAGAGCATCTCTCAGGAAGGCACTCCTGACTTTA  
CTAAATGTGATCCTGGACACATAAAAAACAGCTGTACTTCTACTTTATAATATA  
CGACCATATTGTGAGGAAAAAAAAAAAAAAAAAAAAAA

LJS03 (SEQ ID NO: 52)

TCAGTTAACGAGATTTCAAGCTAAAGAAACTTAACTAAGATGCGATTCCCTTTGGC  
CTTCTCCGTTGCTTGGTCTTCACCAACATTGCCAAACCAGGTCTTGGGACATTGTA  
ACTGGTATTAATGATATGGTAAAAAATACTGCGAATGCACTCAAAATCGTCTAACAC  
5 TTCTGTGACATTATTCAAAATACCATCACCGAAGCTATAAAAATGCAAATTCTCTGT  
TTCGGAACTCCTCAGCAAGTCATGAAACCCCTACGGATATTATTAATGGTGTAGGACA  
AGTGCAGAGTGCCTTGTGAATTCACTGGAAATGTTGTGCAAATTGTTGATGCCGC  
TGGAAATGTTTGGAAAGTTGTTGATGAGGCTGGAAATATCGTGGAGGTAGCTGGAA  
CAGCATTGAAACTATCATTCCACTGCCCGGTAGTGATTCAAGATAATTGATGCTC  
10 TCCAAGGAAATGCAGGGACTACATCGGATTCACTGGCTTCAACTGTGCCCAACAATCT  
TAACTACAACCGCAATGATGTTGCTTAACGGAGAATTAAATTGAATATCAAAAT  
CCAAGATGAAATATTCAAGATTTCAATCAATATGATAACGAAATTGAAATTATTTC  
CGACTAAAGCAATTGAAAAGGAAACCAAATAAATTGAAATTGAAAGAAAAAA  
AAAAA  
15

LJS192 (SEQ ID NO: 54)

ATATCAATTATCATCATGGTGAAGTACTCGTGTCTGTTGCAATTCTTCT  
GGCCGGACCCCTACGGCGTTGAGGTTCTGTGAGAATGACCTGACAGAGGCCAAGT  
ATCTCAAGATGAATGCAATGCAAGGTGAAATTGAGATGAATTCTACCCCTCTGAAG  
20 AAGAAGTGGGTGAAGCATTGAGCGACAAACAGAAAACGTGCAGGAAGTCACCAACAT  
CGTGAGAGGATGCTTGAAGCTGAACAAGCCAAAGAGCATGGAAATGTGAAAGATT  
CCGCTTGAGTCAATGCTACATTGAAAAGAATTATGTCAATTCTCTAAAATATTGAA  
AGAAAAGTTATGAATGAAAATTCTGAAATTGTTGCAAAATATATAAATTGCCA  
ATTAAAA  
25

LJM19 (SEQ ID NO: 56)

AGTTAATTTCATCATGAAGTTCTTACTTGATTTCTCTGCAATTCTTCTGGCTGA  
TCCTGCTTGGTCAAGTGTTCAGAGGATTGTGAGAATATTTCATGACAATGCGTACCT  
CCTTAAATTGGATTGTGAAGCAGGAAGGGTTGATCCTGTGAATACGACGATATTCCGG  
30 ATGAAGAAATATGAAATAACGGTCGATGTTGGAGTTCATCTGAGGACCAGGAGAAA  
GTTGCGAAAATAATAAGGGAGTGCATTGCACAAGTTCAACGCAAGATTGACGAAATT  
TTCAGAAATTATGATTGTTACATGAAGAAGAAAATCTGTAATTATTATCCTGAAAATAT  
GTAAAAAAATTATTATATAAAAAATATAAGGATTAAACGATGAAACACAAGACCAAAAAAAA  
TAAAAATGGCCTAATATTGAAGCAAAATTAAAGCATGAAACACAAGACCAAAAAAAA  
35 AAAA  
AAAAA

LJL138 (SEQ ID NO: 58)

TCAATCTAACATGCACCTGCAATTGAATTGTGCGCTATTCTCCTTCGGTACTAAATG  
GAATTCAAGGGCGCTCCAAAAGTATTAATTCAAAATCCTGCGCAATCTCCTTCGGAGA

ATGTAACGGCTAAGAAGGAGCCAGTGTACTTGAAACCATCAAATGATGGCTATTGAGT  
 ACCCCCCCTACAGCCAAGTGGGCCATTGTAAGTCTCAAATTGGAGAATCTCTGCAATC  
 TTCTGTCCAGGTGATGGAAAGGACGTAGAGACAATTACGTCAATACAAATTTCGATT  
 AGCTTCATATTGTCAACAAGAGCACATCAACGGATACCATTGAAACGGAAGAAGTT  
 5 GCGGAGGAAGTGGAAAAGTGTACAAAGTTGGCTTCCGCTGCCCTCTGGAAATTCCAT  
 TCAATCTACCAAACGTGTTGATAAGAAAAATCTCACACCTCTACTCAATTACACATT  
 CTCAATGGTCAAGCTGTTGGATATCACCTTAAGCACACAAGAGGAAGCTTGTACCAA  
 TGGTATCTACGGAAAGTCAACATTGATAAACTCTACAAGACGCAAATTGAGAAATTCA  
 10 ACAAAACTTTGGCCCTAAACAAACATTTCGGTAGACCCCTCAATTCTATCACGTG  
 TACATTAACACAGCACCACAGTACCAATCAATTATCAAGGAAATTGGCTACGTGTTGA  
 AAATCACGTGAGGGATCTCGCAAAAGTTCTGCAGAAGGACATAACAGTCGTACGGAA  
 TTTGGGGATACTCGGTTGAAGAGTAAGAAAATAGAGAAAGAAATCTATTAGGAGAT  
 GACGTAATTGCCGTACCAAGCAATGTTCTGGAAGGCTGTTTGACCCCTCAAAAACAAGA  
 15 AGCAATTGTCTTGTTCCTCAAATAATCCCCACGTGAAGACCTTAATCCCAACTGCAA  
 GGATGTATCGCTCAAGCTGGATTGGAAATGATAATCTGAATATTCTCCAATTATTCT  
 TATTGGTCTGACTATTGTTGCAAACATTGAGGAATTGTTAAAAGAAATAAAATAATTCT  
 ACCCAAAGAAGTAAATAACAAAAACTACACCAAAAAACTCCTTAAGTTCTAAACAA  
 GAAACAAGGAGGGAGATAAGAAGGTGGTACGTAAGCGGCCAAAGGAGCATAATATT  
 20 AACGAAAAAAAAAAAAAAAAAAAAAA

LJL15 (SEQ ID NO: 60)

GTTCTACGATAAAATTCTTCAAAACTTTCTTTAAAGAAAAATCTCAAAAAGTTA  
 AAATGAATTGCACCTTGCATTATCCTCTTGTGAGTTACTTCACACTGATCACTGCTAC  
 25 GGATCTAATTGAAAAGGAACCTTCTGATTGCAAAAAGATCTCATCTCCAAGGCTGAGC  
 TAACTTGGTCCAAGCTCTGATTCTGTACCGAACAAAACCTAACATTGCTCTCAATT  
 AATCCGCCGGAAAATGATGAGGTGACTAAAGCAGTTCGAGCTGAGGTTATGATCAAGACAAGGATT  
 GACACAAAGAAGTCTCACATTGGCTCGGAGGTATTGTTATGATCAAGACAAGGATT  
 CCGTGGATAAGCGATGGAACAACGTGTTACGAAGACAGTCTACATCAATTGGTACCAAG  
 30 GAGAACCAAATGGTGGAGGTACCAAAAGGAATTGTATGGAATTGTACTTTAAACT  
 CCAGCTGGTCAATGGAATGATGATATTGTACAGCAAAGCATCATTATATGTCAGGA  
 GAAAAAAATAATTGAATTGTTCATGTGTCTTGGCGGTGCGAAGGTATAATTGAGGTTG  
 ACGACATAAATTGATTTCTTCATTAAGAAAATAAGGCTGAATTATAAAAAAAAA  
 AAAAAAAAAAAAAAA

35

LJL91 (SEQ ID NO: 62)

GTTCTACGATAAAATTCTTCAAAACTTTCTTTAAAGAAAAATCTCAAAAAGTTA  
 AAATGAATTGCCCTTGCATTATCCTCTTGTGAGTTACTTCACACTGATCACTGCTGC  
 GGATCTAAGTGAAGGAACTTCTGATGGCAAAAAGATCTCATCTCCAAGGCTGAGC

TAAGTTGGTCGATGCTCTCGATGCCTGTACGAAAAAGACCTAACCTTGCTCACAAATT  
AATCCGCCCGGAAAATGAGGAAGTGACTAAAGCAGTCGAGCTGAGGTTCATCTCCA  
GACACAAAGAAGTCTCACATTGGCTCGGAGGTATTCGTATGATCAAGACAAGGATT  
CCGTTGGATAAGCGATGGAACAACGTACGAAGACAGTCTACATCAATTGGTACCAAG  
5 GAGAACCAAATGGTGGGAGGTACCAAAAGGAATTGTATGGAATTGTACTTAAACT  
CCAGCTGGTCAATGGAATGATGATATTGTACAGCAAAGCATCATTATATGTCAGGA  
GAAAAAAATAAATTGAATTGTCATGTGCTTGGCGGTGCGAAGGTATAATTGAGTTG  
ACGACATAAATTGATTTCTTCATTAAGAAAATAAGGCTGAATTAGCAAAAAAAA  
AAAAA  
10 LJM11 (SEQ ID NO: 64)  
TTGAATTGAAGCAGCAGCAATGAAAGTGTCTCAATTTCACGCTCGCCTCTCCA  
AGGGACCCCTGGAGCGGACTCAAGGATATAATGGAAGCAATTGCTCTACAATAATG  
TTACACCAGGATCCTACAATCCGATAATATGATCAGTACGGCTTGCCTACGATGCTG  
15 AGGGTAAAAAACTCTCCTAGCTGTCCAAGGAAGTTACCCAGAGTTCCGTATACATTG  
CGCGAAGTGGATACAAAGAATAGTCTTGGTGTAAAGGGAAAACATTCACCGTTACTTAA  
CAAATTCACTGGGACAAAACGGAAAGGAACTAACATCAATCTACGCCAGTTATTG  
ATGATTGTCGTCGCCCTTGGTGGTGTATTGGTCCGTGGAATATCGCTCAAGAGGTG  
CCAAAGACTACCCGAGTCATCGTCCCTGCAATTGTCGTACGACCTAAAGCAACCAAAC  
20 TACCCCGAAGTTGTCGATACTATTCCCCACAAGATTAGTGGAGAAGCCAACATATTIC  
GGTGGATTGCCGTTGATGTTGCAAACCCAAAGGGGGATTGTAGTGAACACTTTGTCTAC  
ATTACAAACTCCTCAGGGGAGCTCTTATATACGATCATAAGAACGAGGATTGTCGG  
AATGTAACTCATCCCACCTTCAAAGCAGAACGACCCACTAAATTGATTACGGCGGAAA  
GGAATATGAATTCAAAGCCGAATTTCGGAATTACTCTCGGAGATCGAGACAGTGAAG  
25 GCAATCGCCAGCTACTACTAGCCGGAAGTGCATCAAAGTCTACAGCGTCAACACG  
AAAGAACTTAAGCAGAAGGGTGGAAAGCTGAATCCGGAGCTCTGGAAACCGCGGGA  
AGTACAACGATGCCATTGCCCTAGCTTACGATCCAAAACAAAGTTATCTTCTTGTG  
AGGCCAACACAAAGCAAGTATCCTGCTGGAACACACAGAAAATGCCACTGAGGATGAA  
GAATACCGACGTAGTCTACACTAGTCTCGCTTGTCTTGGAAACGGACATTGCGTTGA  
30 TAGCAAGGGCGGCCTCTGGTTCATGTCTAACGGCTTCCGCCTATAAGGAAATCAGAAA  
AATTCAAATATGACTTCCCACCGCTACCGTCTAATGAGGATCATGGACACACAGGAAGCA  
ATTGCCGGAACTGCTTGCATGATGCAATGCAAAAGTTAATTCAACCCAAAGAAGAAG  
ACCTAAAGAGGCTTCCAGGCTTGTGAGGAGAGGTGGTATCAACGCAAAATCAG  
CTATTGTTGATGAGGAGGAGAAATTATTGATTCTGAATTCTATAAAAAAAATTAAATT  
35 GTGAAATATTGGCAATAATAATTGAATTACAAAAAA  
AAAAAA

LJS138 (SEQ ID NO:66)

TCTCTTGGTTAACATTGTGAAGTTATCGGACGTGGCCGGTTCTATTCTTTGCAAAAA  
TGCAGTCAAAATTCTTCTTCGCTTTCACCTTACCTTGGGCTATGTTTGGGTGA  
AACATGCTCAAATGCTAAGGTTAAGGGAGCTACCTCTTATTCCACAACGGATGCCACAA  
5 TTGTAAGCCAATTGCCTTGTGACTGAATTCTCCTTGGAAATGCTCAAATCCTGGATCCG  
AGAAAATCTCCCTATTGCTGAAGTCGATGGAAAATTACTCCTGTTGCCATGATGGGG  
ATACCACCTACCAGGTGAGCTGGAATGAAGAGGTTAATAAGGCTAGAAGTGGTACTAC  
AGTGTGAAGCTGTACGATGAAGAAGGATACGGAGCAGTACGCAAAGCTCAGAGATCAG  
GTGAAGAGAACAGGTCAAACCACTAGCAACCGTTGTTGACATCCAGGAACATAC  
10 ACTGGACCATGGTTCAATTCCGAAATCCTCGCAGCTGGTCTCATTGCTGTTGCCTAC  
TTGCTTCTCAACCGAAGCAAAATTCTTCTAAAGAGACGCAGCATGAAATTTCACA  
AAAAAATAAAAACAAATTCAAGTCATCAACCATGTCTTTGGCACTCAGACTGTTCTG  
TGAAATACAAACTATTATTAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL124 (SEQ ID NO: 68)

ATCCCCACAAGAACGCTAAATGGTGTCAATTCTGTTAATCTCCTTGATTCTTAATT  
GTTGGTTTCTATGCTAAAGCTAGACCACTAGAACGACATCTCGTCAGATCTTCCCCTGA  
TTATTACATCACTGAAGGCTATGACGGTGTGAAGGAGAACGAGAGATCGAACTTGTAC  
CTGTGACATTGGAATATTAATATACATACAACACCTGCTCCAGAATTACCTTGAAT  
20 GGTAAAAAAATCCAAGAAGAATTATGATTATTCTTCTCCATTGGGATGGATTGTAA  
GTCAGCATAAAACGCCGTTAAAATGAATTTTAATAAAAAAAATTATTCCAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAA

LJL35 (SEQ ID NO: 70)

25 CACTATTGGAAGATTATTAACCTCAAGATGAAATTATTTGTTAATTTGTTGT  
GTTTGTGCTTAGAACGATCTGTATAGAGACCGTGAAAGCTATGGAAGCAACGGAGGAGA  
TATCTGTAAAATTGCAAGATGATGCGAATGAACCTGATGACTCTCTGGATTAGACGAA  
GGTCTCCTGATGCATTGATGAGGACTATAATAATCAGGCTGAGTACAAGCCGAATCC  
TAGAGGGACTACAGAACGATAATTAAATATAAATTCAAGGAAAACACTCTAAAATT  
30 CCAATTGACTCTACTTAAACGATTAAACCTACACTAAATACCATATGCAATAA  
TTATGTTTAATTATTAGTGCAGATCTACTAGTTTCAGTCATATTGGGACTTCCC  
GCCTTCTCGATGGAAAATGATTACGGATTCTTAATTTCATTGTACAGAGTTAAT  
AAAACAATTGAAAGCAATTAAAAAAAAAAAAAAAAAAAAAA

35 Also included are fragments of the above-described nucleic acid sequences that are at least 33 bases, at least 36 bases, at least 42 bases or at least 48 bases in length, which is sufficient to permit the fragment to selectively hybridize to a polynucleotide that encodes a disclosed *Lu. longipalpis* under specified conditions. The term "selectively hybridize" refers to hybridization under moderately or highly stringent conditions, which excludes non-related nucleotide sequences.

Also disclosed herein are open reading frames (ORFs) encoding a *Lu. longipalpis* polypeptide. These ORFs are delimited by a start codon and by a stop codon. This also includes the degenerate variants and nucleotide sequences encoding conservative variants and homologs.

Specific, non-limiting examples of open reading frames are as follows:

5 The L JL34 unprocessed protein is encoded by nucleic acids 30-842 of SEQ ID NO:1, and the mature protein is encoded by the nucleic acid sequence 87-842 of SEQ ID NO:1.

The L JL18 unprocessed protein is encoded by nucleic acids 56-532 of SEQ ID NO:3, and the mature protein is encoded by the nucleic acid sequence 113-532 of SEQ ID NO:3.

10 The L JS193 unprocessed protein is encoded by nucleic acids 216-502 of SEQ ID NO:5, and the mature protein is encoded by the nucleic acid sequence 276-502 of SEQ ID NO:5.

The L JS201 unprocessed protein is encoded by nucleic acids 48-353 of SEQ ID NO:7, and the mature protein is encoded by the nucleic acid sequence 117-352 of SEQ ID NO:7.

The L JL13 unprocessed protein is encoded by nucleic acids 26-766 of SEQ ID NO:9, and the mature protein is encoded by the nucleic acid sequence 83-766 of SEQ ID NO:9.

15 The L JL23 unprocessed protein is encoded by nucleic acids 18-992 of SEQ ID NO:11, and the mature protein is encoded by the nucleic acid sequence 81-992 of SEQ ID NO:11.

The L JM10 unprocessed protein is encoded by nucleic acids 92-571 of SEQ ID NO:13, and the mature protein is encoded by the nucleic acid sequence 149-571 of SEQ ID NO:13.

20 The L JL143 unprocessed protein is encoded by nucleic acids 46-948 of SEQ ID NO:15, and the mature protein is encoded by the nucleic acid sequence 115-948 of SEQ ID NO:15.

The L JS142 unprocessed protein is encoded by nucleic acids 25-507 of SEQ ID NO:17, and the mature protein is encoded by the nucleic acid sequence 85-507 of SEQ ID NO: 17.

The L JL17 unprocessed protein is encoded by nucleic acids 28-342 of SEQ ID NO:19, and the mature protein is encoded by the nucleic acid sequence 88-342 of SEQ ID NO:19.

25 The L JM06 unprocessed protein is encoded by nucleic acids 50-523 of SEQ ID NO:21, and the mature protein is encoded by the nucleic acid sequence 107-523 of SEQ ID NO:21.

The L JM17 unprocessed protein is encoded by nucleic acids 24-1264 of SEQ ID NO:23, and the mature protein is encoded by the nucleic acid sequence 83-1264 of SEQ ID NO:23.

30 The L JL04 unprocessed protein is encoded by nucleic acids 30-914 of SEQ ID NO:25, and the mature protein is encoded by the nucleic acid sequence 81-914 of SEQ ID NO:25.

The L JM114 unprocessed protein is encoded by nucleic acids 29-475 of SEQ ID NO:27, and the mature protein is encoded by the nucleic acid sequence 101-475 of NO:27.

The L JM111 unprocessed protein is encoded by nucleic acids 24-1214 of SEQ ID NO:29, and the mature protein is encoded by the nucleic acid sequence 78-1214 of SEQ ID NO:29.

35 The L JM78mature unprocessed protein is encoded by nucleic acids 42-1091 of SEQ ID NO:31, and the mature protein is encoded by the nucleic acid sequence 102-11091 of SEQ ID NO:31.

The L JS238 unprocessed protein is encoded by nucleic acids 27-206 of SEQ ID NO:33, and the mature protein is encoded by the nucleic acid sequence 87-206 of SEQ ID NO:33.

The LJS169 unprocessed protein is encoded by nucleic acids 11-370 of SEQ ID NO:35, and the mature protein is encoded by the nucleic acid sequence 77-370 of SEQ ID NO:35.

The L JL11 unprocessed protein is encoded by nucleic acids 30-1745 of SEQ ID NO:37, and the mature protein is encoded by the nucleic acid sequence 105-1745 of SEQ ID NO:37.

5 The L JL08 unprocessed protein is encoded by nucleic acids 26-238 of SEQ ID NO:39, and the mature protein is encoded by the nucleic acid sequence 95-238 of SEQ ID NO:39.

The LJS105 unprocessed protein is encoded by nucleic acids 24-275 of SEQ ID NO:41, and the mature protein is encoded by the nucleic acid sequence 81-275 of SEQ ID NO:41.

10 The L JL09 unprocessed protein is encoded by nucleic acids 74-1954 of SEQ ID NO:43, and the mature protein is encoded by the nucleic acid sequence 128-1954 of SEQ ID NO:43.

The L JL38 unprocessed protein is encoded by nucleic acids 40-165 of SEQ ID NO:45, and the mature protein is encoded by the nucleic acid sequence 100-165 of SEQ ID NO:45.

The L JM04 unprocessed protein is encoded by nucleic acids 40-456 of SEQ ID NO:47, and the mature protein is encoded by the nucleic acid sequence 100-456 of SEQ ID NO:47.

15 The L JM26 unprocessed protein is encoded by nucleic acids 96-1616 of SEQ ID NO:49, and the mature protein is encoded by the nucleic acid sequence 147-1616 of SEQ ID NO:49.

The LJS03 unprocessed protein is encoded by nucleic acids 41-553 of SEQ ID NO:51, and the mature protein is encoded by the nucleic acid sequence 98-553 of SEQ ID NO:51.

20 The LJS192 unprocessed protein is encoded by nucleic acids 18-344 of SEQ ID NO:53, and the mature protein is encoded by the nucleic acid sequence 87-344 of SEQ ID NO:53.

The L JM19 unprocessed protein is encoded by nucleic acids 16-360 of SEQ ID NO:55, and the mature protein is encoded by the nucleic acid sequence 82-360 of SEQ ID NO:55.

The L JL138 unprocessed protein is encoded by nucleic acids 12-1238 of SEQ ID NO:57 and the mature protein is encoded by the nucleic acid sequence 72-1238 of SEQ ID NO:57.

25 The L JL15 unprocessed protein is encoded by nucleic acids 63-542 of SEQ ID NO:59, and the mature protein is encoded by the nucleic acid sequence 120-542 of SEQ ID NO:59.

The L JL91 unprocessed protein is encoded by nucleic acids 63-542 of SEQ ID NO:61, and the mature protein is encoded by the nucleic acid sequence 120-542 of SEQ ID NO:61.

30 The L JM11 unprocessed protein is encoded by nucleic acids 20-1216 of SEQ ID NO:63, and the mature protein is encoded by the nucleic acid sequence 74-1216 of SEQ ID NO:63.

The LJS138 unprocessed protein is encoded by nucleic acids 12-1238 of SEQ ID NO:65, and the mature protein is encoded by the nucleic acid sequence 72-138 of SEQ ID NO:65.

The L JL124 unprocessed protein is encoded by nucleic acids 23-241 of SEQ ID NO:67, and the mature protein is encoded by the nucleic acid sequence 83-241 of SEQ ID NO:67.

35 The L JL35 unprocessed protein is encoded by nucleic acids 12-1238 of SEQ ID NO:69, and the mature protein is encoded by the nucleic acid sequence 72-1238 of SEQ ID NO:69.

Another specific non-limiting example of a polynucleotide encoding a *Lu. longipalpis* polypeptide is a polynucleotide having at least 75%, 85%, 90%, 95%, or 99% homology to one of the

sequences set forth above that encodes a polypeptide having an antigenic epitope or function of a *Lu. longipalpis* polypeptide. Yet another specific non-limiting example of a polynucleotide encoding a *Lu. longipalpis* polypeptide is a polynucleotide that encodes a polypeptide that is specifically bound by an antibody that specifically binds the *Lu. longipalpis* polypeptide.

5 The *Lu. longipalpis* polynucleotides include a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (for example, a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of either nucleotide. The term includes  
10 single and double forms of DNA.

Recombinant vectors are also disclosed herein that include a polynucleotide encoding a polypeptide or a fragment thereof according to the disclosure. Recombinant vectors include plasmids and viral vectors and may be used for *in vitro* or *in vivo* expression.

15 A plasmid may include a DNA transcription unit, for instance a nucleic acid sequence that permit it to replicate in a host cell, such as an origin of replication (prokaryotic or eukaryotic). A plasmid may also include one or more selectable marker genes and other genetic elements known in the art. Circular and linear forms of plasmids are encompassed in the present disclosure.

20 For *in vivo* expression, the promoter is generally of viral or cellular origin. In one embodiment, the cytomegalovirus (CMV) early promoter (CMV-IE promoter), including the promoter and enhancer, is of use. The CMV-IE promoter can be of human or murine origin, or of other origin such as rat or guinea pig (see EP 0260148; EP 0323597; WO 89/01036; Pasleau *et al.*, *Gene* 38:227-232, 1985; Boshart M. *et al.*, *Cell* 41:521-530, 1985). Functional fragments of the CMV-IE promoter may also be used (WO 98/00166). The SV40 virus early or late promoter and the Rous Sarcoma virus LTR promoter are also of use. Other promoters include but are not limited to, a  
25 promoter of a cytoskeleton gene, such as (but not limited to) the desmin promoter (Kwissa M. *et al.*, *Vaccine* 18(22):2337-2344, 2000), or the actin promoter (Miyazaki J. *et al.*, *Gene* 79(2):269-277, 1989). When several genes are present in the same plasmid, they may be provided in the same transcription unit or in different units.

30 The plasmids may also comprise other transcription regulating elements such as, for example, stabilizing sequences of the intron type. In several embodiments the plasmids include the first intron of CMV-IE (Published PCT Application No. WO 89/01036), the intron II of the rabbit  $\beta$ -globin gene (van Ooyen *et al.*, *Science* 206:337-344, 1979), the signal sequence of the protein encoded by the tissue plasminogen activator (tPA; Montgomery *et al.*, *Cell. Mol. Biol.* 43:285-292, 1997), and/or a polyadenylation signal (polyA), in particular the polyA of the bovine growth hormone  
35 (bGH) gene (U.S. Patent No. 5,122,458) or the polyA of the rabbit  $\beta$ -globin gene or of SV40 virus.

In a specific, non-limiting example, the pVR1020 plasmid (VICAL Inc.; Luke C. *et al.*, *Journal of Infectious Diseases* 175:91-97, 1997; Hartikka J. *et al.*, *Human Gene Therapy* 7:1205-

1217, 1996)) can be utilized as a vector for the insertion of such a polynucleotide sequence, generating recombinant plasmids.

The plasmids are evaluated in dogs in order to determine their efficacy against a Leishmania infection (Vidor E. *et al.*, P3.14, XXIV World Veterinary Congress, Rio de Janeiro, 5 Brazil, 18-23 August 1991).

Various viral vectors are also of use with a polynucleotide encoding a *Lu. longipalpis* polypeptide. A specific, non-limiting example includes recombinant poxvirus, including avipox viruses, such as the canarypox virus. Another specific, non-limiting example includes recombinant poxvirus, including vaccinia viruses (U.S. Patent No. 4,603,112), such as attenuated vaccinia virus 10 such as NYVAC (see U.S. Patent No. 5,494,807) or Modified Vaccinia virus Ankara (MVA, Stickl H. and Hochstein-Mintzel V., *Munch. Med. Wschr.* 113:1149-1153, 1971; Sutter G. *et al.*, *Proc. Natl. Acad. Sci. USA* 89:10847-10851, 1992; Carroll M. W. *et al.*, *Vaccine* 15(4):387-394, 1997; Stittelaar K. J. *et al.*, *J. Virol.* 74(9):4236-4243, 2000; Sutter G. *et al.*, *Vaccine* 12(11):1032-1040, 1994). When avipox viruses are used, canarypox viruses (U.S. Patent No. 5,756,103) and fowlpox 15 viruses (U.S. Patent No. 5,766,599) are of use, such as attenuated viruses. For recombinant canarypox virus vectors, the insertion sites may be in particular in the ORFs C3, C5 or C6. When the expression vector is a poxvirus, the heterologous polynucleotide can be inserted under the control of a poxvirus specific promoter, such as the vaccinia virus 7.5kDa promoter (Cochran *et al.*, *J. Virology* 54:30-35, 1985), the vaccinia virus I3L promoter (Riviere *et al.*, *J. Virology* 66:3424-3434, 1992), 20 the vaccinia virus HA promoter (Shida, *Virology* 150:451-457, 1986), the cowpox virus ATI promoter (Funahashi *et al.*, *J. Gen. Virol.* 69:35-47, 1988), other vaccinia virus H6 promoter (Taylor *et al.*, *Vaccine* 6:504-508, 1988; Guo *et al.*, *J. Virol.* 63:4189-4198, 1989; Perkus *et al.*, *J. Virol.* 63:3829-3836, 1989).

Other viral vectors of use are herpes virus or adenovirus vectors. Specific, non-limiting 25 examples include a canine herpes virus (CHV) or canine adenovirus (CAV) vector (for example, see U.S. Patent No. 5,529,780; U.S. Patent No. 5,688,920; Published PCT Application No. WO 95/14102). For CHV, the insertion sites may be in particular in the thymidine kinase gene, in the ORF3, or in the UL43 ORF (see U.S. Patent No. 6,159,477). For CAV, the insertion sites may be in 30 particular in the E3 region or in the region located between the E4 region and the right ITR region (see U.S. Patent No. 6,090,393; U.S. Patent No. 6,156,567). In one embodiment in CHV or CAV vectors the insert is in general under the control of a promoter (as described above for the plasmids), such as CMV-IE promoter.

Multiple insertions can be done in the same vector using different insertion sites or using the same insertion site. When the same insertion site is used, each polynucleotide insert is inserted under 35 the control of different promoters. The insertion can be done tail-to-tail, head-to-head, tail-to-head, or head-to-tail. IRES elements (Internal Ribosome Entry Site, see European Patent EP 0803573) can also be used to separate and to express multiple inserts operably linked to the same promoter. Bacterial vectors can also be used for *in vivo* expression.

Any polynucleotide according to the disclosure can be expressed *in vitro* by DNA transfer or expression vectors into a suitable host cell. The host cell may be prokaryotic or eukaryotic. The term "host cell" also includes any progeny of the subject host cell. Methods of stable transfer, meaning that the foreign polynucleotide is continuously maintained in the host cell, are known in the art. Host 5 cells can include bacteria (for example, *Escherichia coli*), yeast, insect cells, and vertebrate cells. Methods of expressing DNA sequences in eukaryotic cells are well known in the art.

As a method for *in vitro* expression, recombinant Baculovirus vectors (for example, *Autographa California Nuclear Polyhedrosis Virus* (AcNPV)) can be used with the nucleic acids disclosed herein. For example, polyhedrin promoters can be utilized with insect cells (for example, 10 *Spodoptera frugiperda* cells, like Sf9 cells available at the ATCC under the Accession number CRL-1711, or Sf21 cells) (see for example, Smith *et al.*, *Mol. Cell Biol.* 3:2156-2165, 1983; Pennock *et al.*, *Mol. Cell Biol.* 4: 399-406, 1994; Vialard *et al.*, *J. Virol.* 64:37-50, 1990; Verne A., *Virology* 167:56-71, 1988; O'Reilly *et al.*, "Baculovirus expression vectors, A laboratory manual," New York Oxford, 15 Oxford University Press, 1994; Kidd I. M. & Emery V.C., "The use of baculoviruses as expression vectors," *Applied Biochemistry and Biotechnology* 42:37-159, 1993; European Patent No. EP 0370573; European Patent No. EP 0265785; U.S. Patent No. 4,745,051). For expression the BaculoGold™ Starter Package (Cat # 21001K) from Pharmingen (Becton Dickinson) can be used.

As a method for *in vitro* expression, recombinant *E. coli* can be used with a vector. For example, when cloning in bacterial systems, inducible promoters such as arabinose promoter, pL of 20 bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter), and the like may be used.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl<sub>2</sub> method using procedures well 25 known in the art. Alternatively, MgCl<sub>2</sub> or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such methods of transduction of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be 30 cotransformed with *Lu. longipalpis* polynucleotide sequences, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector (see above), such as a herpes virus or adenovirus (for example, canine adenovirus 2), to transiently transduce eukaryotic cells and express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982). In addition, 35 a transfection agent can be utilized, such as dioleoyl-phosphatidyl-ethanolamine (DOPE).

Isolation and purification of recombinantly expressed polypeptide may be carried out by conventional means including preparative chromatography (for example, size exclusion, ion exchange, affinity), selective precipitation and ultra-filtration. Such a recombinantly expressed polypeptide is part of the present disclosure. The methods for production of such a polypeptide are

also encompassed, in particular the use of a recombinant expression vector comprising a polynucleotide according to the disclosure and of a host cell.

#### Antibodies

5        A *Lu. longipalpis* polypeptide of the disclosure or a fragment thereof according to the disclosure can be used to produce antibodies. Polyclonal antibodies, antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibodies are included. Such antibodies are of use as markers for exposure, and as immunodiagnostic tools to follow the development of the immune response to *Lu. longipalpis* 10 salivary proteins.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green et al., "Production of Polyclonal Antisera," *Immunochemical Protocols*, pp. 1-5, Manson, ed., *Humana Press*, 1992; Coligan et al., "Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters," *Current Protocols in Immunology*, section 2.4.1, 1992.

15       The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, *Nature* 256:495, 1975; Coligan et al., sections 2.5.1-2.6.7; and Harlow et al., *Antibodies: A Laboratory Manual*, p. 726, Cold Spring Harbor Pub., 1988. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B 20 lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion- 25 exchange chromatography. See, for example, Coligan et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes et al., "Purification of Immunoglobulin G (IgG)," *Methods in Molecular Biology*, Vol. 10, pp. 79-104, *Humana Press*, 1992.

Methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies are well known to those skilled in the art. Multiplication *in vitro* may be carried out in suitable culture media such as 30 Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally supplemented by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, thymocytes, or bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large-scale hybridoma cultivation can be carried out by 35 homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells, for example, syngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils

such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

Antibodies can also be derived from subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in WO 91/11465, 5 1991, and Losman *et al.*, *Int. J. Cancer* 46:310, 1990.

Alternatively, an antibody that specifically binds a polypeptide can be derived from a humanized monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the 10 framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:3833, 1989. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 15 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeyen *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.* 12:437, 1992; and Singer *et al.*, *J. Immunol.* 150:2844, 1993.

Antibodies can be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, p. 119, 1991; Winter *et al.*, *Ann. Rev. Immunol.* 12:433, 1994. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies can be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies 25 in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described 30 by Green *et al.*, *Nature Genet.* 7:13, 1994; Lonberg *et al.*, *Nature* 368:856, 1994; and Taylor *et al.*, *Int. Immunol.* 6:579, 1994.

Antibodies include intact molecules as well as fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv which are capable of binding the epitopic determinant. These antibody fragments retain some ability to selectively bind with their antigen or receptor and are defined as follows:

35 (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain (L) and a portion of one heavy chain(H);

(2) Fab', the fragment of an antibody molecule that can be obtained by treating a whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) (Fab')<sub>2</sub>, the fragment of the antibody that can be obtained by treating a whole antibody with the enzyme pepsin without subsequent reduction; F(ab')<sub>2</sub> is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain (V<sub>L</sub>) and the variable region of the heavy chain (V<sub>H</sub>) expressed as two chains; and

(5) Single chain antibody (SCA), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988).

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')<sub>2</sub>. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulphydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and references contained therein; Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al.*, *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V<sub>H</sub> and V<sub>L</sub> chains. This association may be noncovalent (Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659, 1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See, for example, Sandhu, *supra*. In one embodiment, the Fv fragments comprise V<sub>H</sub> and V<sub>L</sub> chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V<sub>H</sub> and V<sub>L</sub> domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are known in the art (see Whitlow *et al.*, *Methods: a Companion to Methods in*

*Enzymology*, Vol. 2, page 97, 1991; Bird *et al.*, *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271, 1993; and Sandhu, *supra*).

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by 5 constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 106, 1991).

Antibodies can be prepared using an intact polypeptide or fragments containing small 10 peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from substantially purified polypeptide produced in host cells, *in vitro* translated cDNA, or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the peptide include keyhole limpet 15 hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize an animal (for example, a mouse, a rat, or a rabbit).

Polyclonal or monoclonal antibodies can be further purified, for example, by binding to and 20 elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See for example, Coligan *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1991).

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first 25 monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first mono-clonal antibody.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, label (for example, enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

In one embodiment, an antibody that binds a *Lu. Longipalpis* polypeptide can be used to 30 assess whether a subject has been bitten by a sand fly. In one specific, non-limiting example, a sample is obtained from a subject of interest, such as a human or a dog. The sample can be a body fluid (for example, blood, serum, urine, saliva, etc.) or a tissue biopsy. The sample or a fraction thereof is contacted with the antibody, and the ability of the antibody to form an antigen-antibody complex is assessed. One of skill in the art can readily detect the formation of an antigen-antibody 35 complex. For example, ELISA, Western blot, or radio-immune assays can be utilized.

#### Immunogenic Compositions, Vaccines and Methods of Use

Immunogenic compositions and vaccines are disclosed herein. In one embodiment the immunogenic compositions and vaccines include a polypeptide. In another embodiment, the

immunogenic compositions and vaccines include a recombinant vector, such as a viral vector or a plasmid. When administered to a subject such an immunogenic composition or vaccine generates an immune response to the sand fly's salivary protein(s), and surprisingly a reduction of the leishmaniasis symptoms and a decrease of the leishmania parasite load results. Thus, without being bound by theory, a cellular response, such as a Th1 response, produced against the salivary protein can indirectly kill a Leishmania parasite. For example, a Th1 type response can allow macrophages to take up Leishmania antigens and present them to T cells in a Th1 context. The induction the Th1 response can produce an anti-Leishmania immune response, or can prime the immune system of the mammalian host for anti-Leishmania immunity in response to a later infection.

10 In one embodiment, the immunogenic composition or the vaccine includes an effective amount of at least one *Lu. longipalpis* polypeptide disclosed herein. The immunogenic composition and the vaccine can include a pharmaceutically acceptable excipient and/or an adjuvant. In one embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. In one specific, non-limiting example, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67. In specific, non-limiting examples, the immunogenic composition includes a polypeptide having a sequence set forth as one of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59.

In one embodiment, the immunogenic composition includes more than one *Lu. longipalpis* polypeptide, such as two, three, four, five, six, ten or more of the polypeptides disclosed herein. Thus, the immunogenic composition includes at least one polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these

polypeptides, a conservative variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, and optionally another polypeptide having an amino acid sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or SEQ ID NO: 69, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant of one of these polypeptides, or a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides.

In specific non-limiting examples, the immunogenic composition includes an amino acid having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, SEQ ID NO: 39, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant of one of these polypeptides, or a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. Thus, the immunogenic composition can include a polypeptide having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39. These compositions include, but are not limited to, an immunogenic composition including a polypeptide having a sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59, and a polypeptide having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39.

The immunogenic composition or the vaccine can include a pharmaceutically acceptable excipient and/or an adjuvant.

In another embodiment, the immunogenic composition or the vaccine includes an effective amount of at least one *Lu. longipalpis* polypeptide in conjunction with one or more *P. perniciosus* polypeptide(s) and/or one or more *P. ariasi* polypeptide(s). These polypeptide sequences are disclosed in U.S. Patent Application No. 60/412,327, filed September 19, 2002, U.S. Patent Application No. 60/425,852, filed November 12, 2002, and PCT Application No. PCT/US03/29833, filed September 18, 2003, which are incorporated herein by reference.

In one embodiment, the immunogenic composition or the vaccine comprises an effective amount of a recombinant vector expressing at least one *Lu. longipalpis* polypeptide disclosed herein and a pharmaceutically acceptable vehicle or excipient. In one specific, non-limiting example the recombinant vector encodes at least one polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ

14 ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a conservative  
variant, a homolog, an immunogenic fragment, or a fusion protein thereof. In specific non-limiting  
examples the vector encodes a polypeptide having a sequence as set forth as SEQ ID NO: 3, SEQ ID  
NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59, a polypeptide at least  
5 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a  
conservative variant, a homolog, an immunogenic fragment, or a fusion protein thereof. In several  
examples the vector encodes one or more polypeptides having a sequence as set forth as SEQ ID NO:  
3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59. The  
vector can also optionally encode a polypeptide having a sequence as set forth as SEQ ID NO: 1,  
10 SEQ ID NO: 23, or SEQ ID NO: 39.

11 The immunogenic composition can include a nucleic acid sequence encoding a *P. ariasi*  
polypeptide(s) and/or a *P. perniciosus* polypeptide(s) (see U.S. Provisional Application No.  
15 60/412,327, which is incorporated by reference herein in its entirety). In one embodiment, the *Lu.*  
*longipalpis* polypeptide(s) having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID  
NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID  
NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ  
ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47,  
SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO:  
61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a conservative variant, a homolog, an  
20 immunogenic fragment, or a fusion protein thereof, are encoded by the same recombinant vector as a  
*P. ariasi* polypeptide(s) and/or a *P. perniciosus* polypeptide(s). In another embodiment, the *Lu.*  
*longipalpis* polypeptide(s), a *P. ariasi* polypeptide(s) and/or a *P. perniciosus* polypeptide(s), are  
encoded by different recombinant vectors.

25 The *Lu. longipalpis* polypeptide can be administered by any means known to one of skill in  
the art (See Banga, A., "Parenteral Controlled Delivery of Therapeutic Peptides and Proteins,"  
*Therapeutic Peptides and Proteins*, Technomic Publishing Co., Inc., Lancaster, PA, 1995) such as by  
intramuscular (IM), intradermal (ID), subcutaneous (SC), or intravenous injection, but even oral,  
nasal, or anal administration is contemplated. In one embodiment, administration is by subcutaneous,  
intradermal, or intramuscular injection using a needleless injector (Biojector<sup>TM</sup>, Bioject, Oregon,  
30 USA).

35 To extend the time during which the peptide or protein is available to stimulate a response,  
the peptide or protein can be provided as an implant, an oily injection, or as a particulate system. The  
particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar  
particle. (see, for example, Banja, *supra*). A particulate carrier based on a synthetic polymer has  
been shown to act as an adjuvant to enhance the immune response, in addition to providing a  
controlled release. Aluminum salts may also be used as adjuvants to produce a humoral immune  
response. Thus, in one embodiment, a *Lu. longipalpis* polypeptide is administered in a manner to  
induce a humoral response.

In another embodiment, a *Lu. longipalpis* polypeptide is administered in a manner to direct the immune response to a cellular response (that is, a CTL response), rather than a humoral (antibody) response. A number of means for inducing cellular responses, both *in vitro* and *in vivo*, are known. Lipids have been identified as agents capable of assisting in priming CTL *in vivo* against 5 various antigens. For example, as described in U.S. Patent No. 5,662,907, palmitic acid residues can be attached to the alpha and epsilon amino groups of a lysine residue and then linked (for example, via one or more linking residues, such as glycine, glycine-glycine, serine, serine-serine, or the like) to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated in a liposome, or emulsified in an adjuvant. As another example, *E. coli* lipoproteins, 10 such as tripalmitoyl-S-glycercylcysteinylseryl-serine can be used to prime tumor specific CTL when covalently attached to an appropriate peptide (see, Deres *et al.*, *Nature* 342:561, 1989). Further, as the induction of neutralizing antibodies can also be primed with the same molecule conjugated to a peptide which displays an appropriate epitope, the two compositions can be combined to elicit both 15 humoral and cell-mediated responses where that is deemed desirable.

15 In yet another embodiment, an MHC class II-restricted T-helper epitope is added to the polypeptide of the disclosure to induce T-helper cells to secrete cytokines in the microenvironment to activate CTL precursor cells. The technique further involves adding short lipid molecules to retain the construct at the site of the injection for several days to localize the antigen at the site of the injection and enhance its proximity to dendritic cells or other "professional" antigen presenting cells 20 over a period of time (see Chesnut *et al.*, "Design and Testing of Peptide-Based Cytotoxic T-Cell-Mediated Immunotherapeutics to Treat Infectious Diseases and Cancer," Powell, *et al.*, (eds.), *Vaccine Design, the Subunit and Adjuvant Approach*, Plenum Press, New York, 1995).

An immunogenic composition or a vaccine according to the disclosure can be prepared in 25 accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species, and condition of the particular subject, and the route of administration. The immunogenic composition or the vaccine can be administered alone, or in combination with adjuvant(s) and/or with other antigen(s). The other antigen(s) can be a *Leishmania* antigen. In one embodiment, the 30 *Leishmania* antigen is the A2 antigen, such as the A2 antigen from *L. infantum* (see Published PCT Patent Application No. WO 95/06729 and in particular the sequence given in SEQ ID NO:2). The other antigen(s) can be present in the composition as a protein, or as an immunological fragment thereof (for example, an epitope), or as an insert in an expression vector (for example, recombinant viral vector, recombinant plasmid, in particular the pVR1012 (Vical Inc.; Hartikka J. *et al.*, *Human Gene Therapy* 7:1205-1217, 1996)).

35 Any immunogenic composition, vaccine, or therapeutic composition according to the disclosure can be mixed with an adjuvant.

Polypeptide-based compositions:

In several embodiments, the polypeptide-based immunogenic compositions and vaccines according to the disclosure are formulated with (1) vitamin E, saponin (for example, Quil A™, QS21™), aluminum hydroxide, aluminum phosphate, aluminum oxide ("Vaccine Design, The 5 subunit and adjuvant approach," *Pharmaceutical Biotechnology*, vol. 6, Edited by Micheal F. Powell and Mark J. Newman, 1995, Plenum Press New York), (2) an acrylic acid or methacrylic acid polymer, a polymer of maleic anhydride and of alkenyl derivative, (3) an immunostimulating sequence (ISS), in particular an oligodeoxyribonucleotidic sequence bearing one or more non-methylated CpG groups (Klinman D. M. *et al.*, *Proc. Natl. Acad. Sci. USA* 93:2879-2883, 1996; 10 Published PCT Application No. WO 98/16247), (4) to formulate the immunogenic or vaccine preparation in the form of an oil-in-water emulsion, in particular the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" edited by M. Powell and M. Newman, Plenum Press, 1995, and the emulsion MF59 described on page 183 of this same book, (5) cytokines, or (6) combinations or mixtures thereof.

15 The cytokines (5) that can be added to the composition, include, but are not limited to, GM-CSF (granulocyte-macrophage colony stimulating factor) or cytokines inducing Th1 (for example, IL-12). All these cytokines can be added to the composition as a protein or as a vector encoding this cytokine protein. In one embodiment, the cytokines are from canine origin, for example, canine GM-CSF, for which a gene sequence has been deposited at the GenBank database (Accession No. 20 S49738). This sequence can be used to create the vector in a manner similar to what was made in the Published PCT Patent Application No. WO 00/77210.

In one specific, non-limiting example the adjuvant contains two or more of an emulsifier, a micelle-forming agent, and an oil. Suitable emulsifiers, micelle-forming agents, and oils are detailed in U.S. Patent Nos. 5, 585,103; 5,709,860; 5,270,202; and 5,695,770, all of which are incorporated by 25 reference. An emulsifier is any molecule that allows the components of the emulsion to remain as a stable emulsion. Such emulsifiers include polysorbate 80 (Sorbitan-mono-9-octadecenoate-poly(oxy-1,2-ethanediyl); manufactured by ICI Americas, Wilmington, Del.), polysorbate 20, polysorbate 21, polysorbate 40, polysorbate 60, polysorbate 61, polysorbate 85, dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, TEEPOL HB7™, and SPAN 80™ SPAN 85™, ethoxylated fatty alcohols, 30 ethoxylated fatty acids, ethoxylated castor oil (hydrogenated or not). In one embodiment, these emulsifiers are provided in an amount of approximately 0.05 to approximately 0.5%. In another embodiment, these emulsifiers are provided in an amount of approximately 0.2%. A micelle forming agent is an agent which is able to stabilize the emulsion formed with the other components such that a micelle-like structure is formed.

35 Examples of such agents include polymer surfactants described by BASF Wyandotte publications, for example, Schmolka, *J. Am. Oil. Chem. Soc.* 54:110, 1977, and Hunter *et al.*, *J. Immunol.* 129:1244, 1981, PLURONIC™ L62LF, L101, L121, and L64, PEG1000, and TETRONIC™ 1501, 150R1, 701, 901, 1301, and 130R1. The chemical structures of such agents are well known in the art. In one embodiment, the agent is chosen to have a hydrophile-lipophile balance

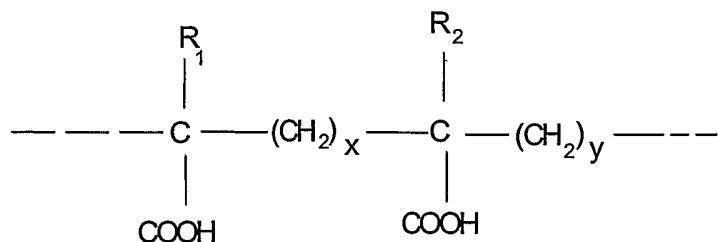
(HLB) of between about 0 and about 2, as defined by Hunter and Bennett, *J. Immun.* 133:3167, 1984. In one embodiment, the agent can be provided in an effective amount, for example between about 0.5 and about 10%. In another embodiment, the agent can be provided in an effective amount, for example between about 1.25 and about 5%.

5 In one embodiment, the oil included in the composition is chosen to promote the retention of the antigen in oil-in-water emulsion, for instance, to provide a vehicle for the desired antigen. In another embodiment, the oil has a melting temperature of less than about 65° C such that emulsion is formed either at room temperature (about 20° C to about 25° C), or once the temperature of the emulsion is brought down to room temperature.

10 The oil-in-water emulsion (4) can be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane, squalene, EICOSANE™ or tetratetracontane; oil resulting from the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of alcohols containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di(caprylate/caprate), glyceryl tri(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. In several embodiments, the emulsifiers are nonionic surfactants, in particular esters of sorbitan, mannide (for example, anhydromannitol oleate), glycerol, polyglycerol, propylene glycol, and oleic, isostearic, ricinoleic, or hydroxystearic acid, which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene 15 copolymer blocks, in particular the Pluronic® products, especially L121. In one specific, non-limiting example, the oil is provided in an amount between about 1 and about 60%. In another specific, non-limiting example, the oil is provided in an amount between about 5 and about 30%. In one embodiment, the adjuvant is a mixture of emulsifiers, micelle-forming agent, and oil available under the name Provax® (IDEC Pharmaceuticals, San Diego, CA).

20 The acrylic acid or methacrylic acid polymers (2) can be cross-linked in particular with polyalkenyl ethers of sugars or of polyalcohols. These compounds are known under the term “carbomer” (*Pharmeuropa*, Vol. 8, No. 2, June 1996). A person skilled in the art may also refer to U.S. Patent No. 2,909,462 (incorporated by reference) describing such acrylic polymers cross-linked with a polyhydroxylated compound containing at least 3 hydroxyl groups. In one embodiment, a 25 polyhydroxylated compound contains not more than 8 hydroxyl groups. In another embodiment, the hydrogen atoms of at least 3 hydroxyls are replaced with unsaturated aliphatic radicals containing at least 2 carbon atoms. In other embodiments, radicals contain from about 2 to about 4 carbon atoms, for example, vinyls, allyls, and other ethylenically unsaturated groups. The unsaturated radicals can themselves contain other substituents, such as methyl. The products sold under the name Carbopol® 30 (Noveon Inc., Ohio, USA) are particularly suitable. They are cross-linked with an allyl sucrose or with allylpentaerythritol. Among these, mention may be made of the products Carbopol® 974P, 934P, and 971P.

The copolymers of maleic anhydride and of an alkenyl derivative, such as the EMA® products (Monsanto) which are copolymers of maleic anhydride and of ethylene, may be linear or cross-linked, for example cross-linked with divinyl ether. Reference may be made to J. Fields *et al.*, *Nature* 186:778-780, 1960 (incorporated by reference). In one embodiment, the acrylic acid or 5 methacrylic acid polymers and the EMA® products are formed from units based on the following formula:



10 in which:

- $R_1$  and  $R_2$ , which may be identical or different, represent H or  $\text{CH}_3$
- $x = 0$  or 1, in one embodiment,  $x = 1$
- $y = 1$  or 2, with  $x + y = 2$ .

For the EMA® products,  $x = 0$  and  $y = 2$ . For the carbomers,  $x = y = 1$ .

15 In one embodiment, the dissolution of these polymers in water leads to an acid solution, which is neutralized to physiological pH, in order to give to the subject the adjuvant solution into which the immunogenic composition or the vaccine itself is incorporated. The carboxyl groups of the polymer are then partly in  $\text{COO}^-$  form.

In one embodiment, a solution of adjuvant, especially of carbomer, is prepared in distilled 20 water. In another embodiment, a solution of adjuvant, especially of carbomer, is prepared in the presence of sodium chloride, the solution obtained being at acidic pH. In another embodiment, this stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with  $\text{NaCl}$ . In yet another embodiment, stock solution is diluted by adding it to the desired quantity of physiological saline ( $\text{NaCl}$  9 g/l) all at 25 once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4). In one embodiment, the stock solution is neutralized with  $\text{NaOH}$ . This solution at physiological pH is used as it is for mixing with the immunogenic composition or with the vaccine, which may be especially stored in freeze-dried, liquid or frozen form.

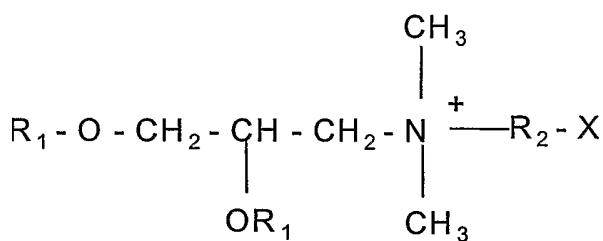
In one embodiment, the polymer concentration in the final vaccine composition is from 30 about 0.01 to about 1.5% W/V. In another embodiment, the final vaccine composition is from about 0.05 to about 1% W/V. In yet another embodiment, the final vaccine composition is from about 0.1 to about 0.4% W/V.

Lipids have been identified as agents capable of stimulating the immune response for various antigens. For example, as described in U.S. Patent No. 5,662,907, palmitic acid residues can be attached to the alpha and epsilon amino groups of a lysine residue and then linked (for example, via one or more linking residues, such as glycine, glycine-glycine, serine, serine-serine, or the like) to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated in a liposome, or emulsified in an adjuvant. As another example, *E. coli* lipoproteins, such as tripalmitoyl-S-glycercylcysteinylseryl-serine, can be used.

To extend the time during which the peptide or protein is available to stimulate a response, the peptide or protein can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (see, for example, Banja, *supra*). A particulate excipient based on a synthetic polymer has been shown to act as an adjuvant to enhance the immune response, in addition to providing a controlled release.

15 Plasmid-based compositions:

In one embodiment, the plasmid-based compositions is formulated with cationic lipids, in particular with cationic lipids containing a quaternary ammonium salt having the following formula:



in which R1 is a saturated or unsaturated linear aliphatic radical from 12 to 18 carbon atoms, R2 is another aliphatic radical comprising from 2 to 3 carbon atoms, and X is an hydroxyl or amine group.

In one embodiment, DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium; Published PCT Application No. WO 96/34109) is the cationic lipid. In another embodiment, the cationic lipid is in association with a neutral lipid, for example DOPE (dioleoyl-phosphatidyl-ethanolamine; Behr J. P., *Bioconjugate Chemistry* 5:382-389, 1994), in order to form the DMRIE-DOPE. In yet another embodiment, the mixture is made extemporaneously about 10 minutes to about 60 minutes before administration. In another embodiment, the mixture is made extemporaneously about 30 minutes before administration. In one embodiment, the molar ratio DMRIE/DOPE is from about 95/5 to about 5/95. In another embodiment, the molar ratio DMRIE/DOPE is about 1/1. In one embodiment, the weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is from about 50/1 to about 1/10. In another embodiment, the weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is from about 10/1 to about 1/5. In yet another embodiment, the weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is from about 1/1 to about 1/2.

In one embodiment, a cytokine or non-methylated CpG groups is added to the composition, as described above for polypeptide-based compositions. The addition can be done advantageously by a plasmid encoding the cytokine.

5           Viral vector-based composition:

The recombinant viral vector-based composition can be supplemented with fMLP (N-formyl-methionyl-leucyl-phenylalanine; U.S. Patent No. 6,017,537) and/or acrylic acid or methacrylic acid polymer adjuvant as described above for polypeptide-based compositions. They can also be formulated with emulsions as described above.

10          In one embodiment, cytokines, non-methylated CpG groups, or emulsions are added to the composition as described above for polypeptide-based compositions. The addition can be done advantageously by a viral vector encoding said cytokine.

15          The immunogenic compositions and vaccines according to the disclosure are conserved and stored either in formulated form at 5°C, or in lyophilized form. In one embodiment, the immunogenic compositions and vaccines according to the disclosure are conserved and stored either in formulated form at 5°C, or in lyophilized form with a stabilizer. Freeze-drying can be done according to well-known standard freeze-drying procedures. The pharmaceutically acceptable stabilizers may be SPGA (sucrose phosphate glutamate albumin) (Bovarnik *et al.*, *J. Bacteriology* 59:509, 1950), carbohydrates (for example, sorbitol, mannositol, lactose, sucrose, glucose, dextran, trehalose), sodium glutamate (Tsvetkov T. *et al.*, *Cryobiology* 20(3):318-23, 1983 ; Israeli E. *et al.*, *Cryobiology* 30(5):519-23, 1993), proteins such as peptone, albumin, or casein, protein containing agents such as skimmed milk (Mills CK *et al.*, *Cryobiology* 25(2):148-52, 1988; Wolff E. *et al.*, *Cryobiology* 27(5):569-75, 1990), and buffers (for example, phosphate buffer, alkaline metal phosphate buffer). An adjuvant may be used to make soluble the freeze-dried preparations.

25

**Methods of Immunization**

The present disclosure provides methods for inducing an immune response to a *Lutzomyia* sand fly polypeptide in a subject. The present disclosure provides further methods for inhibiting or preventing leishmaniasis in a subject.

30          These methods include the administration of at least one immunogenic composition or vaccine according to the disclosure.

35          An immunogenic composition or a vaccine according to the disclosure can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species, and condition of the particular subject, and the route of administration.

If more than one administration is required, they can be administered concurrently (for example, different compositions given during the same period of time via the same or different routes, or a same composition given in the same period of time via different routes), or sequentially

(for example, the same or different compositions given at least two times via the same or different routes). In one embodiment, the delay between two sequential administrations is from about 1 week to about 6 months. In another embodiment, the delay is from about 3 weeks to about 6 weeks. In yet another embodiment, the delay is from about 4 weeks. Following vaccination, annual boost 5 administrations may be done. Advantageously, in a prime-boost vaccination schedule, at least one prime-administration can be done with a composition containing a plasmid according to the disclosure, following by at least one booster administration done with a composition containing a recombinant viral vector according to the disclosure, on the condition that a same *Lu. longipalpis* 10 salivary polypeptide is present twice, coded by the plasmid and by the viral vector. Alternatively, the booster administration can be done with a composition containing a polypeptide according to the disclosure, on the condition that a same *Lu. longipalpis* salivary polypeptide is present twice, coded by the prime-administration plasmid and in the booster polypeptide-based composition.

In such compositions the antigen(s) may be in admixture with a suitable vehicle or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can contain 15 auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling, or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as Remington's Pharmaceutical Science, 17th edition, 1985, incorporated herein by reference, may be consulted to 20 prepare suitable preparations, without undue experimentation. The compositions can also be lyophilized.

Suitable dosages can also be based upon the examples below. For polypeptide-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector™ (Bioject, Oregon, USA). In several embodiments, polypeptide dosages can be 25 from about 1 to 250 µg/ml, from about 15 to about 150 µg/dose, or from about 20 to about 100 µg/dose. In another embodiment, using a needle-less apparatus the volume of a dose can be between about 0.1 ml and about 0.5 ml. In yet another embodiment, using a needle-less apparatus the volume of a dose can be about 0.25 ml. Administration with multiple points of injection is preferred. In one 30 embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.5 to about 1 ml.

For plasmid-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector™. The dosage is from about 50 µg to about 500 µg per 35 plasmid. When DMRIE-DOPE is added, about 100 µg per plasmid is preferred. In one embodiment, when canine GM-CSF or other cytokine is used, the plasmid encoding this protein is present at a dosage from about 200 µg to about 500 µg. In another embodiment, the plasmid encoding this protein is present at a dosage of about 200 µg. In one embodiment, using a needle-less apparatus, the volume of a dose can be between about 0.1 ml and about 0.5 ml. In another embodiment, the volume

of a dose can be about 0.25 ml. In yet another embodiment, administration is performed using multiple points of injection. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, the volumes are from about 0.5 to about 1 ml. The dosage are the same than mentioned above.

5 For recombinant viral vector-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector™. The dosage is from about 10<sup>3</sup> pfu to about 10<sup>9</sup> pfu per recombinant poxvirus vector. In one embodiment, when the vector is a canarypox virus, the dosage is from about 10<sup>5</sup> pfu to about 10<sup>9</sup> pfu. In another embodiment, the dosage is from 10 about 10<sup>6</sup> pfu to about 10<sup>8</sup> pfu. In one embodiment, the volume of needle-less apparatus doses could be between about 0.1 ml and about 0.5 ml. In another embodiment, the volume of needle-less apparatus dose is 0.25 ml. In yet another embodiment, administration is performed using multiple points of injection. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, the volumes are from about 0.5 to 15 about 1 ml. The dosages are the same as mentioned above. In one embodiment, when a syringe with a needle is used, the injection is IM.

20 Advantageously for the prime boost administration regimen, the prime-administration is made with a plasmid-based composition and the boost administration is made with a recombinant viral vector-based composition. In one embodiment, the boost administration is made with a canarypox vector. Both priming and boosting administrations include vectors encoding at least one 25 identical *Lu. longipalpis* salivary antigens, and optionally *Leishmania* A2 antigens. The dosage of plasmids and recombinant viral vectors are the same as above. Optionally, the boost administration can be done with a polypeptide-based composition. In this case, the dosage of polypeptide is from about 1 to about 250 µg/ml, from about 15 to about 150 µg/dose, or from about 20 to about 100 µg/dose.

25 Immunization by nucleic acid constructs is well known in the art and taught, for example, in U.S. Patent No. 5,643,578 (which describes methods of immunizing vertebrates by introducing DNA encoding a desired antigen to elicit a cell-mediated or a humoral response) and U.S. Patent No. 5,593,972 and U.S. Patent No. 5,817,637 (which describe operably linking a nucleic acid sequence 30 encoding an antigen to regulatory sequences enabling expression). U.S. Patent No. 5,880,103 describes several methods of delivery of nucleic acids encoding immunogenic peptides or other antigens to an organism. The methods include liposomal delivery of the nucleic acids (or of the synthetic peptides themselves), and immune-stimulating constructs, or ISCOMS™, negatively charged cage-like structures of 30-40 nm in size formed spontaneously on mixing cholesterol and 35 Quil A™ (saponin). Protective immunity has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-Barr virus-induced tumors, using ISCOMS™ as the delivery vehicle for antigens (Mowat and Donachie, *Immunol. Today* 12:383, 1991). Doses of antigen as low as 1 µg encapsulated in ISCOMS™ have been found to produce class I mediated CTL responses (Takahashi *et al.*, *Nature* 344:873, 1990).

In another approach to using nucleic acids for immunization, a *Lu. longipalpis* polypeptide, or an immunogenic fragment thereof, can also be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, or other viral vectors can be used to express the peptide or protein, thereby eliciting a CTL response. For example, vaccinia vectors and methods useful in immunization protocols are described in U.S. Patent No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the peptides (see Stover, *Nature* 351:456-460, 1991).

In one embodiment, a nucleic acid encoding a *Lu. longipalpis* polypeptide, or an immunogenic fragment thereof, is introduced directly into cells. For example, the nucleic acid may 10 be loaded onto gold microspheres by standard methods and introduced into the skin by a device such as Bio-Rad's Helios™ Gene Gun. A needless injector can also be utilized, such as a Bioinjector2000™. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter. Typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Exemplary dosages for injection are around 0.5 µg/kg to about 50 mg/kg, and 15 typically are about 0.005 mg/kg to about 5 mg/kg (see, for example, U.S. Patent No. 5,589,466). In one embodiment, a prime-boost strategy for immunization is utilized. Thus, in one embodiment, a nucleic acid encoding a *Lu. longipalpis* polypeptide is administered to the subject, followed by immunization with an attenuated or inactivated form of Leishmania.

The immunogenic compositions and the vaccines disclosed herein can be administered for 20 preventative and therapeutic treatments. In therapeutic applications, compositions are administered to a subject suffering from a disease, such as *Leishmaniasis*, in a therapeutically effective amount, which is an amount sufficient to cure or at least partially arrest the disease or a sign or symptom of the disease. Amounts effective for this use will depend upon the severity of the disease and the general state of the subject's health. An effective amount of the compound is that which provides 25 either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In one embodiment, the dosage is administered once as a bolus, but in another embodiment can be applied periodically until a 30 therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the subject.

As noted above, the dosage of the composition varies depending on the weight, age, sex, and method of administration. The dosage can also be adjusted by the individual physician as called for based on the particular circumstances. The compositions can be administered conventionally as 35 vaccines containing the active composition as a predetermined quantity of active material calculated to produce the desired therapeutic or immunologic effect in association with the required pharmaceutically acceptable carrier or diluent (for instance, carrier or vehicle). For example, about 50 µg of a DNA construct vaccine of the present disclosure can be injected intradermally three times

at two week intervals to produce the desired therapeutic or immunologic effect. In another embodiment, a about 1 mg/Kg dosage of a protein vaccine of the present disclosure can be injected intradermally three times at two week intervals to produce the desired therapeutic or immunologic effect.

5 A vaccine is provided herein that includes a *Lu. longipalpis* polypeptide or polynucleotide. Administration of the vaccine to a subject, such as a human or veterinary subject, results in resistance to infection with *Leishmania*. In one embodiment, the subject is a human subject. In another embodiment, the subject is a canine subject, such as a dog.

10 **Methods and Kits for the Diagnosis of *Leishmania* Infection**

It is disclosed herein that individuals who experience an anti-*Leishmania* DTH response conversion also have an increase in antibodies against *Lu. Longipalpis* polypeptide salivary proteins. Thus, the presence or absence of antibodies to *Lu. Longipalpis* polypeptide salivary proteins can be used to ascertain if a subject has a *Leishmania* infection.

15 A method is disclosed herein for diagnosing infection with *Leishmania* by detecting the presence of antibodies that specifically bind one or more polypeptides having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant, a homolog or an immunogenic fragment of one of these polypeptides. The method can utilize a single *Lu. Longipalpis* polypeptide or a combination of these polypeptides. In certain examples, the method of diagnosis detects antibodies that specifically bind at 20 least 3, 6, or 10 of these polypeptides, or immunogenic fragments thereof.

25 In one embodiment, one or more *Lu. Longipalpis* polypeptide can be bound to a solid substrate. For example, the *Lu. Longipalpis* polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 can be bound to the substrate. One or more of these polypeptides can be bound to the substrate, for example at least 3, 30 6, or 10 of these polypeptides, or an immunogenic fragment thereof. In one example, one or more polypeptides having a sequence set forth as one of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59 can be bound to the substrate. In another example, one or more *Lu. Longipalpis* a polypeptides having a sequence set forth as one of SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39 can be bound to the substrate. In one specific, non-

limiting example, at least six *Lu. Longipalpis* polypeptides are bound to a solid substrate, wherein each of the polypeptides comprises an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59, or an immunogenic fragment thereof. In another specific, non-limiting example, at least three *Lu. Longipalpis* 5 polypeptides are bound to a solid substrate, wherein each of the polypeptides comprises an amino acid sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39, or an immunogenic fragment thereof.

In one embodiment, two or more (for example at least 3, 6, or 10) *Lu. Longipalpis* 10 polypeptides (or immunogenic fragments thereof) are applied to a solid substrate, for example as a series of "dots," such as in a "dot blot" assay. In another embodiment, two or more *Lu. Longipalpis* polypeptides are applied to a substrate such as in a linear array. In a further embodiment, *Lu. Longipalpis* polypeptides are applied to a membrane in a two-dimensional array. In this manner, the presence of antibodies to more than one *Lu. Longipalpis* polypeptide is assessed. Each *Lu. Longipalpis* 15 polypeptide can be applied directly to the surface of a membrane in a single location or in a combination of locations.

The solid substrate can be a polystyrene bead, a membrane, a chip or a plate. A plastic or glass substrate can be utilized. In other embodiments, a membrane is utilized that is composed of porous materials such as nylon, nitrocellulose, cellulose acetate, glass fibers, and other porous polymers. The surface of a solid support may be activated by chemical processes that cause covalent 20 linkage of polypeptide to the support. However, any other suitable method may be used for immobilizing a polypeptide to a solid support including, without limitation, ionic interactions, hydrophobic interactions, covalent interactions and the like. Once the polypeptide is applied to the substrate, the substrate can be contacted with a substance, such as protein-containing solution, which non-specifically saturates the binding sites thereon. Specific, non-limiting examples of a protein-containing solution include a solution made from powdered milk or serum albumin, such as bovine 25 serum albumin.

A specimen (for example, sera, blood, plasma, urine, semen, saliva, sputum, lacrimal fluid, lymph fluid) is then added to the substrate, and the combined specimen and substrate are incubated for a sufficient time to allow specific binding. Specific binding of antibodies to the *Lu. Longipalpis* 30 polypeptides disclosed herein, are then detected using any means known to one of skill in the art. In one embodiment, a labeled secondary antibody is used to detect the antibodies that specifically bind the *Lu. Longipalpis* polypeptides. The label can be a radiolabel (for example, <sup>125</sup>I), an enzymatic label (for example, alkaline phosphatase or horseradish peroxidase), or a fluorescent label (for example, fluorescein isothiocyanate). Detection systems for these labels are known to one of skill in 35 the art. Binding of the specimen, or a component of the specimen, to the *Lu. Longipalpis* polypeptide, as indicated by the presence of the marker, indicates infection with *Leishmania*.

In another embodiment, the specimen is adsorbed onto a solid substrate containing binding sites for polypeptides, such as antibody molecules. In one embodiment, the solid substrate is a polystyrene bead, a chip, a membrane or a plate. The substrate is thereafter contacted with a

substance, such as a protein-containing solution that non-specifically saturates the binding sites thereon. The substrate is then washed with a buffer. A solution of one or more *Lu. Longipalpis* polypeptides is then added to the bound specimens. In one embodiment, the *Lu. Longipalpis* polypeptide is directly labeled. The labeling of the *Lu. Longipalpis* polypeptide can be brought about 5 by use of any marker, such as by incorporation of a radioactive isotope or group, or by coupling this component to an enzyme, a dyestuff, for example a chromophoric moiety or a fluorescent group. The enzymes of use are those which can be colorimetrically, spectrophotometrically, or fluorimetrically determined. Non-limiting examples of enzymes for use in the present invention include enzymes from the group of oxidoreductases, such as catalase, peroxidase, glucose oxidase, beta-glucuronidase, 10 beta-D-glucosidase, beta-D-galactosidase, urease and galactose oxidase. After the labeled *Lu. Longipalpis* polypeptide is incubated with the solid substrate, any unbound labeled *Lu. Longipalpis* polypeptide is removed by washing. Bound labeled *Lu. Longipalpis* polypeptide is then detected by an appropriate assay. Binding of the labeled *Lu. Longipalpis* polypeptide to the specimen, or to a component of the specimen, is indicative of infection with *Leishmania*.

15 In general, the incubation steps utilized in carrying out the procedures can be performed in a known manner, such as by incubating at temperatures between about 4° C and about 25° C, for about 30 minutes to about 48 hours. Washings can be included with an aqueous solution such as a buffer, wherein the buffer is from about pH 6 to about pH 8, such as by using an isotonic saline solution of a pH of about 7.

20 Competitive binding assays are also of use in detecting infection with *Leishmania*. One of skill in the art, given the *Lu. Longipalpis* polypeptides disclosed herein, will readily be able to design additional assays, such as competitive binding assays, of use in detecting *Leishmania* infection.

25 In another embodiment, the *Lu. Longipalpis* polypeptides disclosed herein can be included in a diagnostic test kit. For example, a diagnostic test kit for detecting a *Leishmania* infection includes a solid substrate having applied thereon one or more *Lu. Longipalpis* polypeptide disclosed herein. In other embodiments, the kit includes written instructions and/or a container including a specified amount of labeled antibodies to immunoglobulins, such as IgG or IgM, or labeled secondary 30 antibodies that bind antibodies from a species of interest. For example labeled antibodies can be provided that specifically detect dog or human immunoglobulins. The labeled antibodies can be fluorescently labeled, enzymatically labeled, or radiolabeled. Labeled antibodies used in the above-described test kits can be packaged in either solution form, or in lyophilized forms suitable for reconstitution.

35 In another embodiment the test kit includes a specified amount of one or more *Lu. Longipalpis* polypeptide described herein in a container, and written instructions. In one example, the *Lu. Longipalpis* polypeptide is directly labeled. In another example, the one or more *Lu. Longipalpis* polypeptide is unlabeled. If the *Lu. Longipalpis* polypeptide is unlabeled, a container can also be included with a detection reagent that specifically binds the *Lu. Longipalpis* polypeptide, such as a labeled monoclonal antibody. The kit can also optionally include a solid substrate for binding the specimen.

The above described process and test kit for detection of antibodies to the *Lu. Longipalpis* polypeptides disclosed herein can be utilized in many applications, including, but not limited to detecting *Leishmania* infection in a subject using the methods disclosed herein. The tests and kits disclosed herein can be used to detect the efficacy of a therapeutic treatment in a subject. In yet 5 another embodiment, the tests and kits disclosed herein can also be used to assess a primary infection with *Leishmania* or to predict recovery from *Leishmania* infection by taking a body fluid from an infected subject, for example at various times following infection, and applying the above described detection procedures.

10 The disclosure is illustrated by the following non-limiting Examples.

## EXAMPLES

15 **Example 1**  
**Library Construction**

20 *Sand Flies and Preparation of salivary gland homogenate (SGH).* Sand fly Lutzomyia longipalpis salivary glands were obtained from colonized sand flies at Walter Reed Army Institute and at the National Institutes of Health.

Salivary glands dissected under a dissection microscope and collected in microfuge tubes in sterile phosphate buffered saline (PBS), pH 7.0 are stored in dry ice and transferred to -70° C until use.

25 The salivary gland of *Lu. longipalpis* is a sac-like structure consisting of a unicellular epithelium layer surrounding a large lumen (Adler and Theodor, *Ann. Trop. Med. Parasitol.* 20:109, 1926). After a blood meal, the gland total protein content decreases to half or less from its ~1 $\mu$ g value (Ribeiro *et al.*, *Insect Biochem.* 19:409-412, 1989). Accordingly, most of the protein from the fly SGH must be destined for secretion. Indeed, SDS-PAGE of SGH reveals a low complexity composition consisting of ~12 major bands varying from 10-100 kD (Valenzuela *et al.*, *J. Exp. Med.* 194:331-42, 2001). For SDS-PAGE, Tris-glycine gels (16%), 1 mm thick, and NUPAGE 12% BIS-tris gels were used (Invitrogen, Carlsbad, CA). Gels were run with either Tris-glycine or MOPS Nupage running buffer according to the manufacturer's instructions. To estimate the molecular weight of the samples, See BlueJ markers from Invitrogen (myosin, BSA, glutamic dehydrogenase, alcohol dehydrogenase, carbonic anhydrase, myoglobin, lysozyme, aprotinin, and insulin, chain B) 30 were used.

35 SGH were treated with equal parts of 2X SDS sample buffer (8% SDS in Tris-HCl buffer, 0.5M, pH 6.8, 10% glycerol and 1% bromophenol blue dye). Thirty pairs of homogenized salivary glands per lane (approximately 30 $\mu$ g protein) were applied when visualization of the protein bands stained with Coomassie blue was desired. For amino terminal sequencing of the salivary proteins, 40 40 homogenized pairs of glands were electrophoresed and transferred to polyvinylidene difluoride (PVDF) membrane using 10 mM CAPS, pH 11, 10% methanol as the transfer buffer on a Blot-

Module for the XCell II Mini-Cell (Invitrogen, Carlsbad, CA). The membrane was stained with Coomassie blue in the absence of acetic acid. Stained bands were cut from the PVDF membrane and subjected to Edman degradation using a Procise sequencer (Perkin-Elmer Corp, Foster City, CA).

*Salivary Gland cDNA Library Construction.* *Lu. longipalpis* salivary gland mRNA was isolated from 80 salivary gland pairs from adult females. The Micro-FastTrack mRNA isolation kit (Invitrogen, Carlsbad, CA) was used, yielding approximately 100 ng poly (A)+ mRNA. The PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA). One hundred nanograms of *Lu. longipalpis* salivary gland mRNA was reverse transcribed to cDNA using Superscript II RNase H- reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and the CDS/3' primer (Clontech, Palo Alto, CA) for 1 hour at 42° C. Second strand synthesis was performed using a PCR-based protocol by using the SMART III primer (Clontech, Palo Alto, CA) as the sense primer and the CDS/3' primer as anti-sense primer, these two primers additionally, create at the ends of the nascent cDNA SfiI A and B sites respectively. Double strand cDNA synthesis was done on a Perkin Elmer 9700 Thermal cycler (Perkin Elmer Corp., Foster City, CA) and using the Advantage Klen-Taq DNA polymerase (Clontech, Palo Alto, CA). PCR conditions were the following: 94° C for 2 minutes; 19 cycles of 94°C for 10 seconds and 68° C for 6 minutes. Double-stranded cDNA was immediately treated with proteinase K (0.8  $\mu$ g/ $\mu$ l) for 20 minutes at 45° C and washed three times with water using Amicon filters with a 100 kDa cut off (Millipore Corp., Bedford MA). The double-stranded cDNA was then digested with Sfi I for 2 hours at 50° C (The Sfi I sites were inserted to the cDNA during the second strand synthesis using the SMART III and the CDS/3' primer). The cDNA was then fractionated using columns provided by the manufacturer (Clontech, Palo Alto, CA). Fractions containing cDNA of more than 400 base pairs (bp) were pooled, concentrated, and washed three times with water using an Amicon filter with a 100 kDa cut-off. The cDNA was concentrated to a volume of 7  $\mu$ l. The concentrated cDNA was then ligated into a lambda triplex2 vector (Clontech, Palo Alto, CA), and the resulting ligation reaction was packed using the Gigapack gold III from Stratagene/Biocrest (Cedar Creek, TE) following manufacturer's specifications. The obtained library was plated by infecting log phase XL1-blue cells (Clontech, Palo Alto, CA) and the amount of recombinants was determined by PCR using vector primers flanking the inserted cDNA and visualized on a 1.1 % agarose gel with ethidium bromide (1.5  $\mu$ g/ml)

*Massive Sequencing of *Lu. longipalpis* Salivary Gland cDNA Library.*

*Lu. longipalpis* salivary gland cDNA library was plated to approximately 200 plaques per plate (150 mm Petri dish). The plaques were randomly picked and transferred to a 96 well polypropylene plate containing 100  $\mu$ l of water per well. The plate was covered and placed on a gyrator shaker for 1 hour at room temperature. Four microliters of a phage sample was used as a template for a PCR reaction to amplify random cDNAs. The primers used for this reaction were sequences from the triplex2 vector, the primers were named PT2F1 (5'- AAGTACTCT AGCAAT TGTGAGC- 3') (SEQ ID NO:71) which is positioned upstream of the cDNA of interest (5' end), and PT2R1 (5'- CTCTTCGCTATTACGCCAGCT G- 3') (SEQ ID NO:72) which is positioned downstream of the

cDNA of interest (3' end). Platinum Taq polymerase (Gibco-BRL, Gaithersburg, MD) was used for these reactions. Amplification conditions were the following: 1 hold of 75° C for 3 minutes, 1 hold of 94° C for 3 minutes and 34 cycles of 94° C for 30 seconds, 49° C for 30 seconds and 72° C for 1 minute and 20 seconds. Amplified products were visualized on a 1.1% agarose gel with ethidium bromide. Clean PCR was used as a template for a cycle sequencing reaction using the DTCS labeling kit from Beckman Coulter Inc. (Fullerton, CA). The primer used for sequencing (PT2F3) (5'-TCTCGGGAAAGCGCGCCATTGTGTT - 3') (SEQ ID NO:73) is upstream of the inserted cDNA and downstream of the primer PT2F1. Sequencing reaction was performed on a Perkin Elmer 9700 thermacycler. Conditions were 75° C for 2 minutes, 94° C for 4 minutes, and 30 cycles of 96° C for 20 seconds, 50° C for 20 seconds and 60° C for 4 minutes.

After cycle sequencing the samples, a cleaning step was done using the multi-screen 96 well plate cleaning system from Millipore (Bedford, MA). The 96 well multi-screening plate was prepared by adding a fixed amount (according to the manufacturer's specifications) of Sephadex-50 (Amersham Pharmacia Biotech, Piscataway, NJ) and 300  $\mu$ l of deionized water. After 1 hour of incubation at room temperature, the water was removed from the multi screen plate by centrifugation at 750 g for 5 minutes. After the Sephadex in the multi-screen plate was partially dried, the whole cycle sequencing reaction was added to the center of each well, centrifuged at 750 g for 5 minutes and the clean sample was collected on a sequencing microtiter plate (Beckman Coulter, Fullerton, CA). The plate was then dried on Speed-Vac SC 110 model with a microtiter plate holder (Savant Instruments Inc, Holbrook, NY). The dried samples were immediately resuspended with 25  $\mu$ l of deionized ultrapure formamide (J.T. Baker, Phillipsburg, NJ), and one drop of mineral oil was added to the top of each sample. Samples were sequenced immediately on a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) or stored at -30° C. The entire cDNA of selected genes was fully sequenced using custom primers using a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) as described above.

*DNA Vaccine Construction and Description of the VR1020 Vector.* The genes coding for the predicted secreted proteins were amplified from *Lu. longipalpis* specific cDNA by PCR using Platinum Taq polymerase (GIBCO BRL, Gaithersburg, MD) and specific primers carrying the Predicted N-terminus (Forward primer); and the stop codon (Reverse primer) of the selected cDNA. The PCR product was immediately cloned into the custom made VR-2001-TOPO (derived from VR1020 vector) cloning vector following manufacturers specifications (Invitrogen, Carlsbad, CA). The ligation mixture was used to transform TOP10 cells (Invitrogen, Carlsbad, CA) and the cells were incubated overnight at 37° C. Eight colonies were picked and mixed with 10  $\mu$ l of sterile water. Five microliters of each sample were transferred to Luria broth (LB) with ampicillin (100  $\mu$ g/ml) and grown at 37° C. The other 5  $\mu$ l were used as a template for a PCR reaction using two vector-specific primers from the PCRII vector to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.1% agarose gel, the eight PCR products were completely sequenced as described above using a CEQ2000 DNA sequencing

instrument (Beckman Coulter). Cells containing the plasmid carrying the selected *Lu. longipalpis* gene were grown overnight at 37°C on Luria broth with ampicillin (100 µg/ml), and plasmid isolation was performed using the Wizard Miniprep kit (Promega, Madison, WI). The VR-2001-TOPO (a variant of the VR1020 plasmid from Vical) plasmid contains a kanamycin resistance gene, the human cytomegalovirus promoter, and the tissue plasminogen activator signal peptide upstream of the TOPO TA cloning site. The sample that contained the sequence from the start codon to the stop codon in the right orientation and in the correct open-reading-frame following the nucleotide sequence encoding the tissue plasminogen activator signal peptide was chosen.

5 Plasmids were transformed into the TOP-10 strain of *E. coli* (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The transformed bacteria were grown in LB medium and the plasmid was subsequently purified using the commercial plasmid purification kit Megaprep (Qiagen, Valencia, CA). Each plasmid was named according to the name of the polypeptide. Thus pLJL34 is a plasmid encoding LJL34, and pLJM11 is a plasmid encoding LJM11 polypeptide, etc.

10 *Study population.* Sera used in the study using human subjects were obtained from an epidemiologic survey of visceral leishmaniasis (VL) in children less than 7 years of age in an endemic region of São Luiz, Maranhão State, in northeastern Brazil. During this prospective study, anti-*Leishmania* DTH and serology were performed twice a year during 1997 and 1998. Only children who had neither VL, a positive serology, nor DTH on the first survey were included in the study. None of the individuals in the data set had the disease, and all had negative responses to 15 leishmanial antigen during the preceding 6-month period. Positivity in the anti-leishmanial tests reported here indicates a recent conversion determined by a sensitive and specific ELISA (Barral *et al.*, *Am J Trop Med Hyg* 62:740–5, 200) and/or DTH test (Barral *et al.*, *ibid*). To determine the cut-off value for IgG anti-*Lu. longipalpis* in ELISA assays, sera were obtained from children in the same age range from a nonendemic area. Assuming that recent seroconversion represents infection and 20 that a positive DTH response is a marker of protection against leishmaniasis in subclinical cases, we classified children in two groups according to their anti-*Leishmania* responses: Group I, positive 25 serology (S<sup>-</sup> → S<sup>+</sup>) (*n* = 15) and Group II, positive DTH (DTH<sup>-</sup> → DTH<sup>+</sup>) (*n* = 15).

30 *Anti-sand fly saliva serology.* Anti-sand fly saliva serology ELISA was performed as previously described (Barral *et al.*, *ibid*). Sera IgG subclasses were determined using anti-human IgG1, IgG3, or IgG4 alkaline-phosphatase conjugates (Sigma-Aldrich, St. Louis, MO). To determine IgE levels, sera were previously absorbed using Rheumatoid Factor. Anti-human IgE (Sigma-Aldrich, St. Louis, MO) was used in the ELISA.

35 *Western blots.* Western blots of salivary gland antigens were performed as previously described (Barral *et al.*, *ibid*).

35 *Statistical analysis (human studies).* The non-parametric paired Wilcoxon test was used to compare levels of anti-*Lu. longipalpis* saliva antibodies in the same children at time 0 (beginning of survey) and after 6 months. *P* value < 0.05 was established as the significance level. Graph Pad Prism software (San Diego, CA) was used to perform the statistical tests.

**Example 2****DNA and Predicted Protein Sequence Analysis.**

DNA data derived from the mass sequencing project were analyzed by an in-house program  
5 written in VisualBASIC (Microsoft). This program removed vector and primer sequences from the raw sequence. Stripped sequences were compared to the NCBI non-redundant protein database using the program BlastX using the BLOSUM-62 matrix (Altschul *et al.*, *Nucleic Acids Research* 25:3389, 1997). DNA sequences were clustered by blasting the database against itself with a preselected threshold cutoff, usually  $1e^{-10}$  (BlastN program) (Altschul *et al.*, *Nucleic Acids Research* 25:3389, 10 1997). Sequences from the same cluster were aligned using ClustalX (Jeanmougin *et al.*, *Trends Biochem. Sci.* 23:403, 1998). To find the cDNA sequences corresponding to the amino acid sequence obtained by Edman degradation of the proteins transferred to PVDF membranes from SDS-PAGE gels, a search program was written that checked these amino acid sequences against the three possible protein translations of each cDNA sequence obtained in the mass sequencing project. This was 15 written using the same approach used in the BLOCKS (Henikoff *et al.*, *Bioinformatics* 15:471, 1999) or Prosite (Bairoch, *Nucleic Acids Res.* 19 (Suppl.):2241, 1991) programs. Protein translations of the full-length clones were further processed to identify the predicted signal peptides using the Signal P program (Nielsen *et al.*, *Protein Eng.* 10:1, 1997), available online. Predicted signal peptide cleaved sites were compared to the N-terminus sequence obtained from Edman degradation of *Phlebotomus* 20 salivary proteins. Estimation of isoelectric point and molecular weight of translated protein was performed using the DNA STAR program (DNASTAR). Full-length translated protein sequence information was compared with the non-redundant protein database of NCBI using the BLAST-P program (Altschul *et al.*, *Nucleic Acids Research* 25:3389, 1997) and searched for motifs by submitting each sequence to the electronic database.

25 To characterize the primary structure of the main proteins of *Lu. longipalpis* SGH, SDS-PAGE gels were transferred to PVDF membranes, and the amino terminal sequence of each cut band by Edman degradation were estimated.

In addition, the following values were ascertained:

Table 1  
Protein Characteristics

Polypeptide name	Position of cleavage site	Molecular Weight (MW) of Unprocessed Protein	pI of Unprocessed Protein	Molecular Weight of Processed Protein	pI of Processed Protein
LJL34	19	31	9.14	28.9	9.1
LJL18	19	18.7	6.42	16.4	6.1
LJS193	20	34.5	6.59	32.2	6.3
LJS201	23	11.2	4.89	8.7	4.8
LJL13	19	28.7	5	26.6	4.9
LJL23	21	37.4	9.15	35.1	9.1
LJM10	19	18.8	8.73	16.7	8.6
LJL143	23	35	8.4	32.5	8.3
LJS142	20	18.9	6.43	16.7	6.5
LJL17	20	12.3	4.36	10.2	4.4
LJM06	19	18.6	8.79	16.5	8.7
LJM17	18	47.3	5.92	45.2	5.7
LJL04	17	31.1	10.1	29.3	10
LJM114	24	17	7.58	14.3	5.6
LJM111	18	45.2	4.9	43	4.9
LJM78	20	39.4	7.54	37.3	7.7
LJS238	20	6.9	7.92	4.8	6.7
LJS169	22	14.1	4.64	11.6	4.5
LJL11	24	63.4	6.49	60.8	6.7
LJL08	23	9.5	8.76	7	8.8
LJS105	19	9.5	4.85	7.4	4.7
LJL09	18	73	5.65	71.1	5.6
LJL38	20	4.8	3.66	2.5	3.3
LJM04	20	16.2	8.91	13.9	9
LJM26	17	50.7	5.77	48.8	5.8
LJS03	19	17.3	4.27	15.2	4.2
LJS192	23	12.1	4.29	9.7	4.2
LJM19	22	13.4	4.26	10.8	4.2
LJL138	19	45.9	9.42	43.8	9.5
LJL15	19	18.7	6.2	16.5	6.1
LJL91	19	18.5	5.82	16.4	5.8
LJM11	24	45.3	9.35	42.7	9.4
LJS138	20	18.5	5.88	16.2	5.5

### Example 3

#### Antibodies against *Lu. longipalpis* saliva

It has previously been shown that sera from children living in an area endemic for VL have 10 anti-SGS IgG antibodies that differentially recognize salivary gland antigens. Individuals with a positive anti-*Leishmania* DTH response exhibited anti-*Lu. longipalpis* saliva antibodies. A positive correlation was observed between anti-*Lu. longipalpis* saliva antibodies and anti-*Leishmania* DTH, but no correlation was observed between anti-saliva antibodies and anti-*Leishmania* serology (Barral *et al.*, *ibid*).

The change in humoral and cell-mediated anti-*Leishmania* responses in a 6-month follow up of individuals in an area endemic for VL as well as the change in anti-*Lu. longipalpis* saliva antibody responses in the same individuals was studied. Individuals ( $n = 15$ ) who converted to positive anti-*Leishmania* DTH significantly increased their anti-*Lu. longipalpis* IgG (FIG. 1A;  $P = 0.02$ ) and IgE antibody levels (FIG. 1B,  $P = 0.002$ ). IgG1 was the principal antibody subclass involved in the increase of anti-saliva antibodies in the group converting anti-*Leishmania* DTH ( $n = 15$ ) (FIG. 1C); no significant changes were observed in other IgG subclasses. The cut-off value for anti-*Lu. longipalpis* IgG in ELISAs was 0.045. A significant decrease in anti-saliva IgG antibody levels ( $P = 0.035$ ) was observed in sera from children who converted their anti-*Leishmania* serology (Group I) (FIG. 1A). No significant changes were observed in anti-saliva IgE in Group I (FIG. 1B). Although IgG anti-saliva levels in Group II children decreased in the 6-month period, a significant increase in IgG4 anti-saliva was observed in this group ( $P = 0.0245$ ; FIG. 1D).

The number and pattern of *Lu. longipalpis* salivary proteins recognized by the sera of individuals who converted either from  $S^- \rightarrow S^+$  or from  $DTH^- \rightarrow DTH^+$  was evaluated by Western blot. From seven randomly selected sera of individuals who converted their anti-*Leishmania* serology, two poorly recognized two different salivary proteins of 33 kDa and 200 kDa, respectively (FIG. 2A, lane 4); the remaining sera did not recognize any salivary protein at any time point. Conversely, from 13 randomly selected sera of  $DTH^- \rightarrow DTH^+$  individuals, 12 recognized a variety of salivary proteins with various intensities. FIGS. 2A and 2B show the diversity of salivary antigens recognized by these sera (lanes 7-14). Additionally, sera from six  $DTH^- \rightarrow DTH^+$  individuals showed an increase in the number and/or intensity of salivary proteins recognition when comparing time 0 (-) and 6 months (+) time points (FIG. 2A, lanes 7(-) and 8(+), 11(-) and 12(+), 13(-) and 14(+); FIG. 2B, lanes 11(-) and 12(+), 13(-) and 14(+), and data not shown). Some individuals in the  $DTH^- \rightarrow DTH^+$  group did not show any change from time 0 to 6 months (FIG. 2A, lanes 9(-) and 10(+); FIG. 2B, lanes 7(-) and 8(+)) or did not recognize any salivary protein (FIG. 2B, lanes 9(-) and 10(+)).

The sera of the  $DTH^- \rightarrow DTH^+$  individuals recognized a total of 16 different salivary proteins; however, the frequency of recognition varies among these individuals (FIG. 2C). A salivary protein of 45 kDa was recognized by 12 sera, followed by proteins of 44 and 43 and 35 kDa recognized by 8 sera (each), a protein of 17 kDa by 6 sera, and a protein of 16 kDa by 5 sera. Other salivary proteins were recognized as well but with less frequency (3 sera or less, FIG. 2C).

Thus, Group II children, who convert their anti-*Leishmania* DTH, also present an increase in anti-sand fly saliva antibodies as evidenced by ELISA and Western blot. A correlation between anti-saliva antibody titers and anti-*Leishmania* DTH has been shown (Barral *et al., ibid*); the results presented herein show that development of anti-parasite DTH temporally coincides with development of anti-*Lu. longipalpis* saliva antibodies. Without being bound by theory, neutralization of sand fly salivary component(s) by antibodies or cellular response to salivary proteins allows for a more efficient mounting of an anti-*Leishmania* cell-mediated immune response, probably by developing a

Th1 response against the parasite. Sand fly saliva components, such as maxadilan, are able to impair macrophage function (Charlab *et al.*, *Proc Natl Acad Sci USA* 96:15155–60, 1999), which interferes with *Leishmania* survival and antigen presentation (Soares *et al.*, *J. Immunol.* 160:1811–6, 1998). The higher antibody levels observed in DTH<sup>−</sup> → DTH<sup>+</sup> individuals suggest that mounting an 5 immune response against anti-saliva components is linked to developing cell-mediated immunity against *Leishmania*.

The results presently reported by Western blot analysis showed that individuals who converted their anti-*Leishmania* serology practically did not recognize any salivary protein whereas 10 individuals who converted their anti-*Leishmania* DTH recognized a number of different salivary proteins. Frequency of salivary antigens recognized by these sera reveals a cluster of only a few 15 proteins, including antigens with an approximate molecular mass of 45, 44, 43, 35, 27 and 16 kDa (FIG. 2C).

Among these antigens, the recognition of at least two salivary proteins (45 kDa and 35 kDa), 15 represent two of the highest frequencies of recognition by human sera. Surprisingly, only two sera recognized a protein in the range of 6 kDa, the molecular weight of maxadilan (Titus and Ribeiro, *Parasitol Today* 6:157–159, 1990) suggesting that, in humans, maxadilan may not induce a strong antibody response, although it could be a strong inducer of cellular immunity.

Individuals who converted their anti-*Leishmania* cell-mediated immunity exhibited 20 increased IgG1 and IgE levels. IgG1 has been related to a human Th1 response. Elevation of IgE antibodies suggests the development of an immediate hypersensitivity, since IgE is considered a marker of Th2-type responses. Without being bound by theory, it is likely that a mixed Th2-type 25 (related to immediate hypersensitivity) and Th1-like response (related to DTH) against saliva components coexist in individuals who recently converted their anti-*Leishmania* DTH. In fact, this type of mixed response was reported in individuals exposed to insect bites, where the host immune response against insect saliva starts with DTH response and evolves to a predominant immediate-type hypersensitivity and finally desensitization (Melanby, *Nature*. 158, 554–555.13, 1946).

As disclosed herein, in mice, immunization using *Lu. longipalpis* salivary genes resulted in a 30 typical DTH and/or antibody response to *Lu. longipalpis* salivary proteins (see below), suggesting that *Lu. longipalpis* bites could induce Th1 and Th2 responses in humans. Of interest, the *P. papatasii* (SP15) salivary protein responsible for the DTH response in mice is highly homologous to the SL1 protein present in *Lu. longipalpis* saliva (Charlab *et al.*, *Proc Natl Acad Sci USA* 96:15155–60, 1999). Without being bound by theory, the results presented herein suggest that a mixed anti-saliva response with both Th1 and Th2 components can help in establishing an anti-immune 35 *Leishmania* response.

35

#### Example 4

#### DNA Vaccination in Mice

For genetic immunization, Swiss Webster mice were purchased from Taconic Farms. Mice were maintained in the NIAID Animal Care Facility under pathogen-free conditions. Mice were

inoculated in the right ear with 30  $\mu$ g of the plasmid encoding the selected cDNA from *Lu. longipalpis* suspended in 5  $\mu$ l of PBS. Each group is boosted 2 weeks later using the same regimen. Mice were challenged on the opposite ear with salivary gland homogenate of *Lu. longipalpis* and delayed type hypersensitivity (DTH) response is measured 24 hours after the injection by measuring thickness and redness of ear (++: at least 2 mice with a good DTH response, +++ : at least three mice had a good DTH response).

Table 2  
DTH Response in Mice

10

<i>Lutzomyia longipalpis</i> salivary gland cDNA	DTH response (thickness and redness)
pLJS201	-
pLJM19	++
pLJL91	-
pLJM06	-
pLJL15	+++
pLJM11	-
pLJM17	+++
pLJL11	-
pLJL08	++
pLJL18	++
pLJS142	-
pLJL13	-
pLJL34	++
pLJM111	+++
pLJL17	+++
pLJM04	-
pLJL23	++

15

\*Delayed type hypersensitivity (DTH) response induced by injection of salivary gland homogenate on the ear of mice (group of three) previously immunized with salivary DNA vaccine. Mice were previously sensitized with specific DNA plasmids two times at two weeks interval then injected with salivary gland homogenate of the sand fly *Lutzomyia longipalpis*. DTH response was measured at 24 hours (thickness and redness of ear) after salivary gland homogenate injection. (++= at least 2 mice had good DTH response, +++ at least three mice had a good DTH response).

20

### Example 5

#### Production of an Immune Response in Dogs

In a first experiment DTH (delayed type hypersensitivity) reaction is performed in dogs with natural immunity against the leishmaniasis in order to determine which *Lu. longipalpis* salivary proteins are recognized by a protective immune response. These dogs with natural immunity survived without symptoms after two years of exposure in an endemic area. In a second experiment naive dogs are immunized with the *Lu. longipalpis* salivary gland protein expressed by a plasmid in order to evaluate the capability to induce a cellular immune response measured by DTH.

Twelve dogs approximately three years old with natural immunity against Leishmaniasis are injected via an intradermal route (ID) in the back after shaving, with 100  $\mu$ g of each individual plasmid suspended in 100  $\mu$ l of PBS. Each plasmid is injected at a different point. The points are separated by at least 3 cm to avoid interference between DTH responses. The negative control (100  $\mu$ l of buffer) is also inoculated by ID route.

The DTH response is assessed 72 hours after injection by measuring the larger diameter of the skin tumefaction area. The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter greater than or equal to 4 mm at 72 hours after injection.

10 In a second study, 10 naïve dogs 4 to 6 months old are immunized by ID injection in 10 points (100  $\mu$ l per point) in the right ear with a pool of the plasmids encoding a *Lu. longipalpis* polypeptide, 100  $\mu$ g for each one suspended in 1000  $\mu$ l of PBS. On day 21, dogs are injected in 10 points (100  $\mu$ l per point) in the left ear and in 10 points (100  $\mu$ l per point) in the belly with a pool of the plasmids, 100  $\mu$ g for each one suspended in 2000  $\mu$ l of PBS. All dogs are challenged on day 35  
15 by inoculation by ID route in the back (after shaving), with 100  $\mu$ g of each individual plasmid suspended in 100  $\mu$ l of PBS. Each plasmid is injected at a different point. The points are separated by at least 3 cm to avoid interference. As a negative control, 100  $\mu$ l of buffer is inoculated intradermally. The DTH response is assessed 72 hours after challenge, by measuring the larger diameter of the skin tumefaction area. The results are expressed as the mean value of the tumefaction  
20 area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter higher or equal of 4 mm at 72 hours after injection.

The results of this study show that plasmids can induce a cellular immunity in dogs after injection, a cellular immunity revealed by a DTH response. The variation of the DTH response level can be by the variation of the expression of the insert.

25

It will be apparent that the precise details of the methods described may be varied or modified without departing from the spirit of the described disclosure. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

## CLAIMS

We claim:

- 5        1. A substantially purified salivary *Lu. longipalpis* polypeptide.
2. The polypeptide of claim 1, wherein the polypeptide comprises
  - a) an amino acid sequence at least 80% identical to an amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67;
  - b) a conservative variant of the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67;
  - c) an immunogenic fragment comprising at least eight consecutive amino acids of the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, that specifically binds to an antibody that specifically binds the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, respectively; or
  - d) the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, and wherein administration of the polypeptide to a subject produces an immune response to *Lu. longipalpis*.

3. The *Lu. longipalpis* polypeptide of claim 2, wherein the polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or a conservative variant thereof.

10 4. The *Lu. longipalpis* polypeptide of claim 3, wherein the polypeptide comprises an amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67.

5 5. An antigenic fragment of the polypeptide of claim 4.

15 6. The polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence at least 80% identical to the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67.

20 7. An isolated nucleic acid encoding the polypeptide of claim 1.

25 8. The nucleic acid of claim 7, wherein the nucleic acid comprises a sequence as set forth as SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, or SEQ ID NO: 68, or a degenerate variant thereof.

30 9. The nucleic acid of claim 7, wherein the nucleic acid comprises a sequence as set forth as SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16,

SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, or SEQ ID NO: 68.

5

10. The nucleic acid of claim 7, wherein the nucleic acid encodes an amino acid sequence at least 80% identical to the amino acid sequence set forth as SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, or SEQ ID NO: 68.

15

11. The nucleic acid of claim 7, operably linked to an expression control sequence.

15

12. The nucleic acid of claim 11, wherein the expression control sequence is a promoter.

13. The nucleic acid of claim 12, wherein the promoter is an inducible or constitutive promoter.

20

14. The nucleic acid of claim 13, wherein the promoter is a cytomegalovirus promoter.

25

15. A vector comprising the nucleic acid of claim 7.

25

16. The vector of claim 15, wherein the vector is a plasmid.

17. The vector of claim 15, wherein the vector is a viral vector.

18. A host cell transformed with the vector of claim 15.

30

19. An antibody that specifically binds the polypeptide of claim 1.

20. The antibody of claim 19, wherein the antibody is a monoclonal antibody.

35

21. The antibody of claim 20, comprising a detectable label.

22. The antibody of claim 21, wherein the label is a fluorescent, enzymatic or radioactive label.

23. A pharmaceutical composition comprising the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

24. A pharmaceutical composition comprising the nucleic acid of claim 7 and a pharmaceutically acceptable carrier.

25. A method for inducing an immune response to a *Lu. longipalpis* polypeptide in a subject, comprising:

10 administering to the subject a therapeutically effective amount of:

a) an amino acid sequence at least 80% identical to an amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67;

15 b) a conservative variant of the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67;

20 c) an immunogenic fragment comprising at least eight consecutive amino acids of the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, that specifically binds to an antibody that specifically binds the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, respectively; or

35 d) the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49,

SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67; or

(e) a polynucleotide encoding the polypeptide set forth in (a), (b), (c), or (d), thereby inducing the immune response to the *Lu. longipalpis* polypeptide in a 5 subject.

26. The method of claim 25, wherein the immune response is a T cell response.

27. The method of claim 25, wherein the immune response is a B cell response.

28. The method of claim 25, wherein the subject is a non-human veterinary subject.

29. The method of claim 25, wherein the subject is a dog.

15 30. The method of claim 25, wherein the subject is a human.

31. A method for inhibiting a symptom of a *Leishmania* infection or preventing a *Leishmania* infection in a subject, comprising administering to the subject a therapeutically effective amount of:

20 a) an amino acid sequence at least 80% identical to an amino acid sequence set  
forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID  
NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ  
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25 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67;

b) a conservative variant of the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67;

35 c) an immunogenic fragment comprising at least eight consecutive amino acids of the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, that specifically binds to an antibody that specifically binds the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID

NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, 5 respectively; or

10 d) the amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, 15 SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67; or

20 e) a polynucleotide encoding the polypeptide set forth in (a), (b), (c), or (d); thereby inhibiting the symptom of the *Leishmania* infection or preventing the *Leishmania* infection.

15 32. Use of a composition comprising the polypeptide of claim 1 or a nucleic acid encoding the polypeptide of claim 1 for the manufacture of a medicament.

20 33. Use of the composition of claim 32, in conjunction with a composition comprising a *P. ariasi* or a *P. perniciosus* polypeptide for the manufacture of a medicament.

34. A method of diagnosing *Leishmania* infection in a subject, comprising:  
25 contacting a solid substrate comprising at least three, six, or ten polypeptides, wherein each of the polypeptides comprises one amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or an immunogenic fragment 30 thereof;

35 contacting the solid substrate with a sample obtained from the subject; and detecting binding of a component of the sample to at least one polypeptide on the solid substrate, wherein detection of binding of the component to the substrate indicates that the subject is infected with *Leishmania*, thereby diagnosing *Leishmania* infection in the subject.

35. The method of claim 34, wherein one of the at least three polypeptides comprises an amino acid sequence as set forth as SEQ ID NO: 1, wherein another of the at least three polypeptides

comprises an amino acid sequence as set forth as SEQ ID NO: 23, and wherein a further of the at least three polypeptides comprises an amino acid sequence as set forth as SEQ ID NO: 39.

36. The method of claim 34, wherein the solid substrate comprises at least six polypeptides,  
5 wherein a first polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 3, wherein a second polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 11, wherein a third polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 19, wherein a fourth polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 29, wherein a fifth polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 55, and wherein a sixth  
10 polypeptide comprises an amino acid sequence as set forth as SEQ ID NO: 59.

37. The method of claim 34, wherein the solid substrate comprises a polystyrene bead, a chip, a membrane, or a plate.

15 38. The method of claim 34, wherein detecting comprises contacting the component bound to the solid substrate with a labeled secondary antibody.

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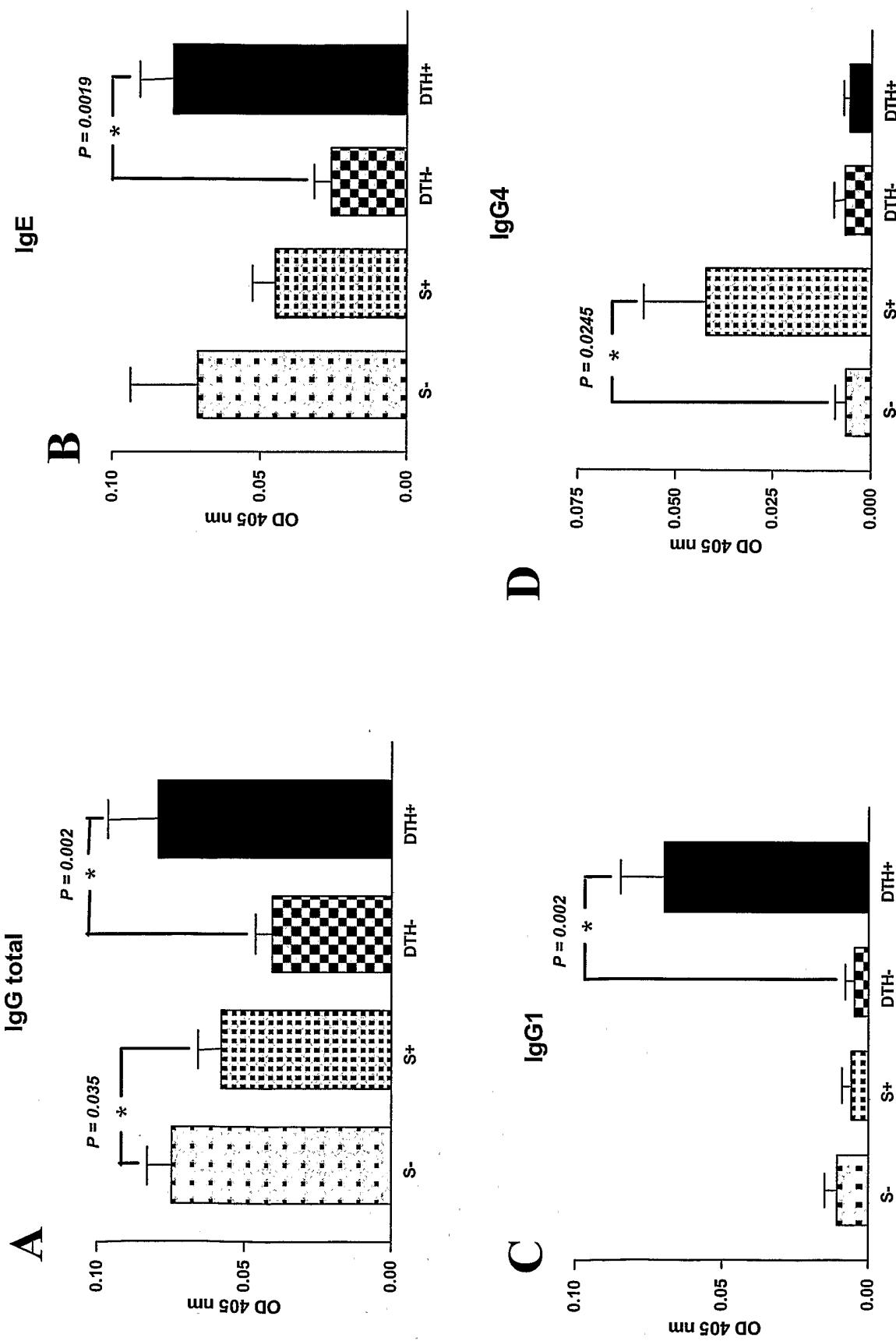


FIG. 1

2/2

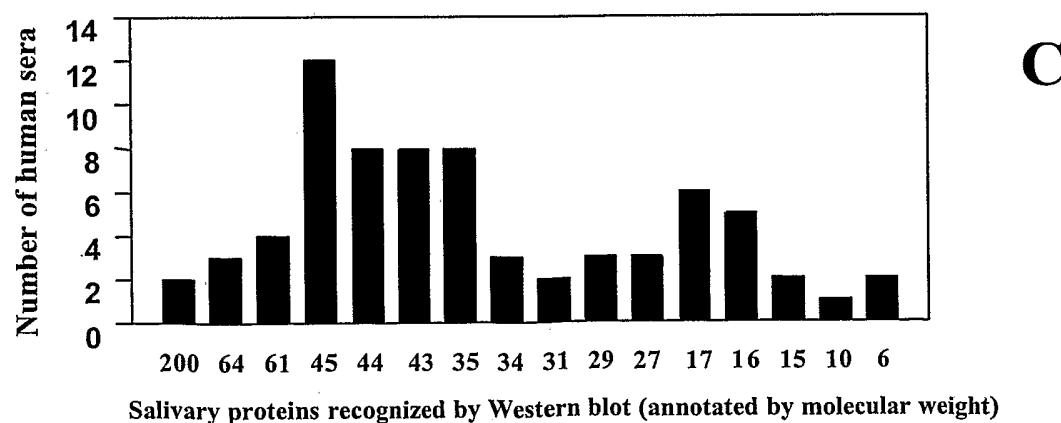
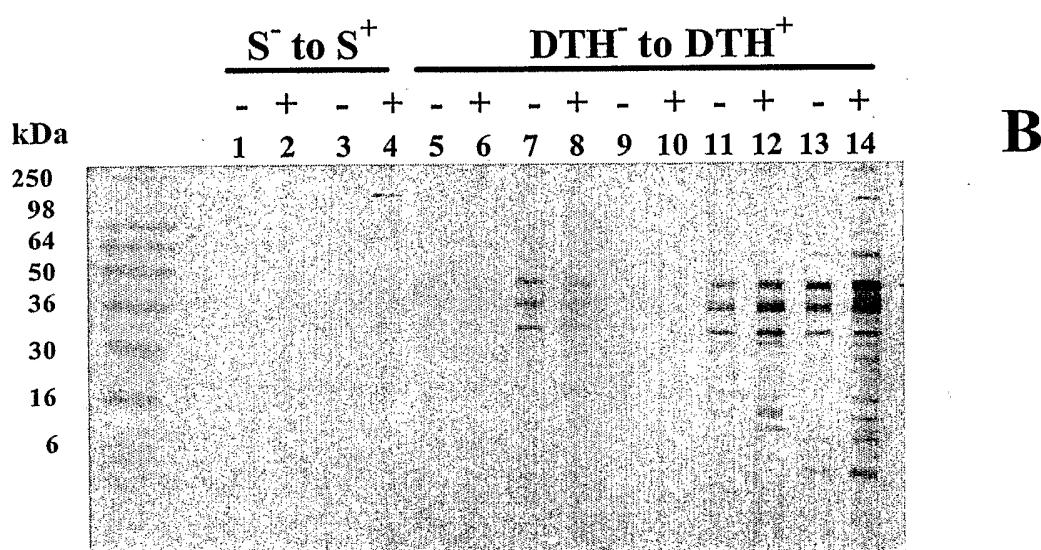
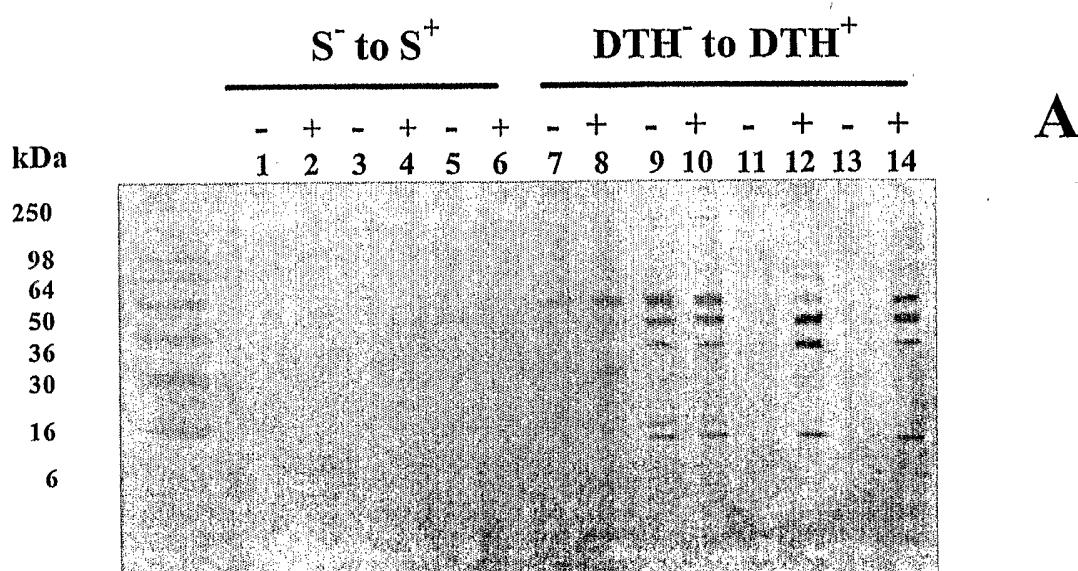


FIG. 2

**STATEMENT ACCOMPANYING SEQUENCE LISTING**

The sequence listing does not include matter that goes beyond the disclosure in the international application.

The printout of the attached Sequence Listing is identical to the computer readable sequence listing on the enclosed computer disk.

## SEQUENCE LISTING

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REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND  
HUMAN SERVICES

Valenzuela, Jesus G.  
Ribeiro, Jose M.C.  
Barral, Aldina  
Netto, Manoel  
Brodklyn, Claudia  
Gomes, Regis

<120> LUTZOMYIA LONGIPALPIS POLYPEPTIDES AND METHODS OF USE

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 gttccaagct cttaatcatt gtgttaaaaa tggctacaca atgggtgtcaa ttaagacatt 240  
 tgaagagaat aaagaactcc ttaaagaact caaaagggtg attaggacag aagatacaca 300  
 agtttggatt ggaggcctca aacatcatca attgcaaac ttgcgttggg taagcgatgg 360  
 aagccacgta gcaacagctt cagggtacac caattgggcc ccaggggagc cagctgattc 420  
 ctcttattac gatcaatttt gcatggcgat gtgttcaga aaagacggcg ctccgtggg 480  
 tgatttgaat tggtgggtta agaatctttt tggttgtag aaacgagatg attgagaggc 540  
 tattttgtt atctcaccgt ttgttgaat aaaaaagaag aagaaagaca aaaaaaaaaaa 600  
 aaaaaaaaaa aaaaaaaaaa 617

<210> 5  
 <211> 304  
 <212> PRT  
 <213> *Lutzomyia longipalpis*

<400> 5

Met Lys Leu Leu Gln Ile Ile Phe Ser Leu Phe Leu Val Phe Phe Pro  
 1 5 10 15

Thr Ser Asn Gly Ala Leu Thr Gly Asn Glu Ser Ala Ala Asn Ala Ala  
 20 25 30

Pro Leu Pro Val Val Leu Trp His Gly Met Gly Asp Ser Cys Cys Phe  
 35 40 45

Pro Phe Ser Leu Gly Ser Ile Lys Lys Leu Ile Glu Gln Gln Ile Pro  
 50 55 60

Gly Ile His Val Val Ser Leu Lys Ile Gly Lys Ser Leu Ile Glu Asp  
 65 70 75 80

Tyr Glu Ser Gly Phe Phe Val His Pro Asp Lys Gln Ile Gln Glu Val  
 85 90 95

Cys Glu Ser Leu Gln Asn Asp Leu Thr Leu Ala Asn Gly Phe Asn Ala  
 100 105 110

Ile Gly Phe Ser Gln Gly Ser Gln Phe Leu Arg Gly Leu Val Gln Arg  
 115 120 125

Cys Ser Ser Ile Gln Val Arg Asn Leu Ile Ser Ile Gly Gly Gln His  
 130 135 140

Gln Gly Val Phe Gly Leu Pro Tyr Cys Pro Ser Leu Ser Arg Lys Thr  
 145 150 155 160

Cys Glu Tyr Phe Arg Lys Leu Leu Asn Tyr Ala Ala Tyr Glu Lys Trp  
 165 170 175

Val Gln Lys Leu Leu Val Gln Ala Thr Tyr Trp His Asp Pro Leu Asn  
 180 185 190

Glu Asp Ala Tyr Arg Thr Gly Ser Thr Phe Leu Ala Asp Ile Asn Asn  
 195 200 205

Glu Arg Gln Ile Asn Asn Asp Tyr Ile Asn Asn Ile Arg Lys Leu Asn  
 210 215 220

Arg Phe Val Met Val Lys Phe Leu Asn Asp Ser Met Val Gln Pro Ile  
 225 230 235 240

Glu Ser Ser Phe Phe Gly Phe Tyr Ala Pro Gly Thr Asp Thr Glu Val  
 245 250 255

Leu Pro Leu Lys Gln Ser Lys Ile Tyr Leu Glu Asp Arg Leu Gly Leu  
 260 265 270

Gln Ser Val Pro Ile Asp Tyr Leu Glu Cys Gly Gly Asp His Leu Gln  
 275 280 285

Phe Thr Lys Glu Trp Phe Ile Lys Phe Ile Ile Pro Tyr Leu Lys Gln  
 290 295 300

<210> 6  
 <211> 1273  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 6  
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attttatgtg cagttttac taaaaatccc ttatcagcaa cccgggtgtct acagtttgt 180  
 cacgctcagt agcatcttca aggtggtaag aaaaaatgaa actcctgcaa atcatcttct 240  
 ctctcttcct ggtcttttc ccgacctaag atggggccct gaccggaaat gaaagtgcag 300  
 caaatgcagc tcccttgccct gtcgtcctgt ggcacgggat gggcgattct tgctgcttc 360  
 ctttcagtt gggaaagcata aaaaaattaa ttgaacaaca aattcctggg attcatgttg 420  
 ttagcctgaa aattggaaag tctctcattt aggactatga aagtggattt tttgttcatc 480  
 cagacaagca aattcaggaa gtttgtgagt cacttcagaa cgatctaaca ctcgcaaatg 540  
 gattcaatgc aattggattt tctcaggta gtcagttcct gcgaggctt gtgcaacgat 600  
 gttcttctat acaagtaagg aatctcattt ccattggagg acagcatcaa ggggttttg 660  
 gtctgcccta ttgtccttcg ttgagcagaa agacttgcga atactttaga aagctcctga 720  
 attatgcagc ttatgaaaaa tgggtacaga aactcctagt tcaagccacc tactggcatg 780  
 atcctctaaa tgaggatgca tatcggactg gaagcacttt cttgctgat ataaataatg 840  
 agagacaaat caataatgac tatattaata atattcgaa gctaaatcgt tttgtgatgg 900  
 taaagttcct caacgacagc atggttcagc caattgaatc tagtttctt ggattctacg 960  
 ctccaggaac tgatacagaa gttctccat taaaacaaag caagattt ttggaagatc 1020  
 gtttggact tcaatcagta ccgatagatt atctagaatg cggaggagat catttgcatt 1080  
 ttacaaaaga atggttcata aagtttatca taccctatct gaagcaataa gagctgcaat 1140  
 gtaattgatt aaaaaatgtt aaccattca ggatgattgg gtgaccctt aaaaatataa 1200  
 atgaaaaaat atacaaaaga aataaatttt tatattgatc ccacaaaaaa aaaaaaaaaa 1260  
 aaaaaaaaaa aaa 1273

<210> 7  
 <211> 102  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 7

Met Arg Asn Phe Ala Val Val Ser Leu Ala Val Ala Val Leu Leu Phe  
 1 5 10 15

Cys Ala Trp Pro Ile Asn Ala Glu Asp Asn Glu Glu Val Gly Lys Ala  
 20 25 30

Arg Glu Lys Arg Gly Leu Lys Asp Ala Met Glu His Phe Lys Asn Gly  
 35 40 45

Phe Lys Glu Leu Thr Lys Asp Phe Lys Leu Pro Ser Leu Pro Ser Leu  
 50 55 60

Pro Gly Phe Gly Lys Lys Pro Glu Ser Gly Ser Ser Glu Asp Ser Gly  
 65 70 75 80

Asp Lys Thr Glu Asp Thr Ser Gly Ser Lys Asp Asp Gln Ser Lys Asp  
 85 90 95

Asn Thr Val Glu Glu Ser  
 100

<210> 8  
 <211> 466  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 8  
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 ataatgaaga agttggaaag gcgagagaaa aaagaggctt aaaagacgca atggaacact 180  
 tcaaaaatgg atttaaggag ctgacaaagg actttaaact tccaaggcctt ccaagtcttc 240  
 ctggatttgg taaaaagcct gaatctggaa gttctgaaga ttctggagat aaaactgagg 300  
 ataccagtgg atctaaggac gaccaatcaa aggataatac ggtcgaagaa tcttaagaaa 360  
 ggcgcaaata gctatttca aagtggcgaa tggttcttgc tttatctgaa ataaatattt 420  
 tttaaaccttt cgaaacccaaa aaaaaaaaaa aaaaaaaaaa aaaaaa 466

<210> 9  
 <211> 247  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 9

Met Asn Phe Leu Leu Lys Ile Phe Ser Leu Leu Cys Leu Cys Gly Leu  
 1 5 10 15

Gly Tyr Ser Trp Gln Asp Val Arg Asn Ala Asp Gln Thr Leu Trp Ala  
 20 25 30

Tyr Arg Ser Cys Gln Lys Asn Pro Glu Asp Lys Asp His Val Pro Gln  
 35 40 45

Trp Arg Lys Phe Glu Leu Pro Asp Asp Glu Lys Thr His Cys Tyr Val  
 50 55 60

Lys Cys Val Trp Thr Arg Leu Gly Ala Tyr Asn Glu Asn Glu Asn Val  
65 70 75 80

Phe Lys Ile Asp Val Ile Thr Lys Gln Phe Asn Glu Arg Gly Leu Glu  
85 90 95

Val Pro Ala Gly Leu Asp Gln Glu Leu Gly Gly Ser Thr Asp Gly Thr  
100 105 110

Cys Lys Ala Val Tyr Asp Lys Ser Met Lys Phe Phe Lys Ser His Phe  
115 120 125

Met Asp Phe Arg Asn Ala Tyr Tyr Ala Thr Tyr Asp Gly Ser Asp Glu  
130 135 140

Trp Phe Ser Lys Asn Pro Asp Val Lys Pro Lys Gly Thr Lys Val Ser  
145 150 155 160

Glu Tyr Cys Lys Asn Lys Asp Asp Gly Asp Cys Lys His Ser Cys Ser  
165 170 175

Met Tyr Tyr Tyr Arg Leu Ile Asp Glu Asp Asn Leu Val Ile Pro Phe  
180 185 190

Ser Asn Leu Pro Asp Tyr Pro Glu Asp Lys Leu Glu Glu Cys Arg Asn  
195 200 205

Glu Ala Lys Ser Ala Asn Glu Cys Lys Ser Ser Val Ile Tyr Gln Cys  
210 215 220

Leu Glu Asn Ala Asp Lys Ser Ala Leu Asp Ala Ser Leu Asn Ile Leu  
225 230 235 240

Asp Glu Phe Ser Gly Arg Tyr  
245

<210> 10  
<211> 955  
<212> DNA  
<213> Lutzomyia longipalpis

<400> 10  
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tctctgtgga ctggggattt catggcagga tgtgagaaat gccgatcaaa ccctctggc 120  
gtatagatcg tgccaaaaga atcctgaaga taaggatcac gtacctcaat ggaggaagtt 180  
cgaattaccc gacgatgaaa agactcattg ctacgtcaag tgcgtatgga cgcgtttggg 240

agcttacaat	gaaaatgaaa	atgtttcaa	aattgatgtc	attactaagc	aatttaatga	300
acgtggccta	gaagttccgg	ctggacttga	tcaagaattg	ggtggttcta	cagatggaac	360
ttgcaaagca	gtttacgata	aatccatgaa	gttcttcaa	tctcattttt	tggactttag	420
gaatgcttac	tacgcaactt	atgacggttc	tgatgaatgg	tttagcaaga	accctgatgt	480
aaaaccgaaa	ggaacaaaag	tttccgaaaata	ctgcaaaaaat	aaagatgtg	gagattgcaa	540
acattcctgc	agtatgtact	actaccgctt	aatcgatgaa	gacaacttag	ttattccgtt	600
cagcaactta	cctgactatc	ccgaagataa	gctcgaggaa	tgcaggaatg	aagccaagtc	660
cgcaaatgag	tgcaaatcat	ctgttatcta	tcagtgtttg	gaaaatgcgg	ataagtcagc	720
tttagacgcg	tcttgaata	tactcgatga	gttttctgga	agatattaaa	acaaactgga	780
taaaaaactt	aggccaacct	atgattcgaa	cttacgattt	tgaacttgaa	attcatgtgc	840
tttaacctat	tgtcccacta	ggaagaaaaa	tccatatttg	gtgatgttaa	actattttg	900
aacctcttca	aaataaaacaa	tttcaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaa	955

&lt;210&gt; 11

&lt;211&gt; 325

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 11

Met	Phe	Leu	Lys	Trp	Val	Val	Cys	Ala	Phe	Ala	Thr	Val	Phe	Leu	Val
1					5						10				15

Gly	Val	Ser	Gln	Ala	Ala	Pro	Pro	Gly	Val	Glu	Trp	Tyr	His	Phe	Gly
						20				25				30	

Leu	Ile	Ala	Asp	Met	Asp	Lys	Lys	Ser	Ile	Ala	Ser	Asp	Lys	Thr	Thr
							35			40				45	

Phe	Asn	Ser	Val	Leu	Lys	Ile	Asp	Glu	Leu	Arg	His	Asn	Thr	Lys	Thr
							50			55				60	

Asp	Gln	Tyr	Ile	Tyr	Val	Arg	Ser	Arg	Val	Lys	Lys	Pro	Val	Ser	Thr
							65			70				75	80

Arg	Tyr	Gly	Phe	Lys	Gly	Arg	Gly	Ala	Glu	Leu	Ser	Glu	Ile	Val	Val
								85			90				95

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

Arg Ile Thr Lys Asp Gly Lys Leu Phe Pro Trp Val Ile Leu Ala Asp  
115 120 125

Ala Asp Gly Gln Arg Pro Asp Gly Phe Lys Gly Glu Trp Ala Thr Ile  
130 135 140

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

Ser Ser Leu Trp Val Lys Lys Ile Thr Lys Asp Gly Val Val Thr Ser  
165 170 175

His Asp Trp Thr Asp Lys Tyr Arg Lys Ile Leu Lys Ala Leu Asn Met  
180 185 190

Pro Asn Gly Phe Val Trp His Glu Ala Val Thr Trp Ser Pro Phe Arg  
195 200 205

Lys Gln Trp Val Phe Met Pro Arg Lys Cys Ser Arg His Pro Phe Ser  
210 215 220

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

Glu Asn Phe Asn Asp Ile Gln Val Ile His Ile Gln Asp Gln Pro Tyr  
245 250 255

Asn Leu Ala Ser Gly Phe Ser Ser Phe Arg Phe Ile Pro Gly Thr Lys  
260 265 270

Asn Glu Arg Leu Leu Ala Leu Arg Thr Val Glu Gln Glu Asp Gln Val  
275 280 285

Lys Thr Trp Ala Val Val Met Asp Met Lys Gly Thr Val Leu Met Tyr  
290 295 300

Glu Lys Glu Leu Tyr Asp Glu Lys Phe Glu Gly Leu Ala Phe Phe Gly  
305 310 315 320

Gly Ile Lys Lys Asn  
325

<210> 12  
<211> 1071  
<212> DNA  
<213> Lutzomyia longipalpis

<400> 12  
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 ctgatatgga caaaaaatcc atcgcgagtg acaaaaaccac cttaacagc gtcctaaaga 180  
 tcgatgaatt gcgccacaac acaaaaacgg atcaatacat ttatgtgcgt agtcgagtga 240  
 agaagccgt ttccacgagg tatgggttca aaggacgcgg tgccgaaattg tcggaaattg 300  
 ttgtcttcaa caataaaactt tacacagttt atgataaaatc tggaaattacg ttccgcataa 360  
 cggaaagacgg aaaactcttc ccgtgggtta ttctcgcaga tgccgatgga cagcgaccgg 420  
 atggcttaa gggtgaatgg gctacaatta aggatgatac aatctatgtt ggatctacgg 480  
 ggatgctcaa gttcacttca tcccttggg tgaagaagat cacgaaagat ggcgttgtta 540  
 cgagtcacga ttggactgat aaataccgaa agattctcaa agctctaaac atgccaaatg 600  
 gtttgcgt gcatgaggct gttacgtggt ctccattcag gaagcaatgg gtcttcatgc 660  
 cgagaaaatg ctcaaggcat cccttcac aggaactcga agaacgcaca gggtgcaata 720  
 aaatagtgac ggcagatgag aatttcaacg acattcaagt tattcacatt caagatcagc 780  
 catataattt agttctggt ttctcttcct tccgctttat tcctggtagc aaaaatgaaa 840  
 gacttctcgc cttgaggaca gtagagcagg aagatcaggt taaaacttgg gctgtggtca 900  
 tggatatgaa aggaacagtt ctgatgtacg aaaaggaact ttatgacgaa aaattcgaag 960  
 gtttagcatt cttgggtggt attaaaaaga attaatttgt tccagaagct tttagatgaa 1020  
 ataataaattt ttatccatt ttaaaaaaaaaaaaaaaaaaaaaaaa a 1071

<210> 13

<211> 160

<212> PRT

<213> *Lutzomyia longipalpis*

<400> 13

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

Ser Thr Ala Leu Gln Val Thr Glu Lys Glu Leu Ser Asp Gly Lys Lys  
 20 25 30

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

Cys Ile His Arg Gly Leu Thr Leu Leu Ser Ile Lys Ser Ala Lys Glu  
 50 55 60

Asn Val Asp Val Thr Lys Ala Ile Arg Ala Glu Leu Asn Phe Asp Ser  
 65 70 75 80

Lys Lys Leu Ala His Val Trp Thr Gly Gly Ile Arg His Ser Gln Asp  
 85 90 95

Lys Tyr Phe Arg Trp Ile Asn Asp Gly Thr Lys Val Val Lys Arg Val  
 100 105 110

Tyr Thr Asn Trp Phe Thr Gly Glu Pro Asn Asn Gly Tyr Trp Lys Asp  
 115 120 125

Glu Phe Cys Leu Glu Ile Tyr Tyr Lys Thr Glu Glu Gly Lys Trp Asn  
 130 135 140

Asp Asp Lys Cys His Val Lys His His Phe Val Cys Gln Glu Lys Lys  
 145 150 155 160

<210> 14

<211> 648

<212> DNA

<213> Lutzomyia longipalpis

<400> 14

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 tctaaagctgc ttgcataatga gcacggcact acaagttact gagaaggaac tttctgtatgg 180  
 gaaaaaagatc ttcatctcca aagttgagct aaactggttc gaagctcttg atttctgtat 240  
 ccatcgtggc ttacgttgc tctcaattaa atccgccaag gaaaatgttag acgtaacaaa 300  
 agcaattcgg gctgaattga attttgattc aaagaaattt gctcatgtgt ggactggagg 360  
 tattcgccat agtcaagata agtatttccg ttggataaat gatggacta aagttgtttaa 420  
 acgagtctac accaattggc tcactggaga accaaataat ggttactgga aggtatgtt 480  
 ttgtctggaa atttactata aaaccgaaga agggaaagtgg aatgtatgata aatgtcacgt 540  
 gaagcatcat tttgtatgtc aagaaaagaa ataaattgtat tgattttgtt tgctgatttg 600  
 cagttcagaa ttgaaaagcc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 648

<210> 15

<211> 301

<212> PRT

<213> Lutzomyia longipalpis

<400> 15

Met Asn Ser Ile Asn Phe Leu Ser Ile Val Gly Leu Ile Ser Phe Gly  
 1 5 10 15

Phe Ile Val Ala Val Lys Cys Asp Gly Asp Glu Tyr Phe Ile Gly Lys  
20 25 30

Tyr Lys Glu Lys Asp Glu Thr Leu Phe Phe Ala Ser Tyr Gly Leu Lys  
35 40 45

Arg Asp Pro Cys Gln Ile Val Leu Gly Tyr Lys Cys Ser Asn Asn Gln  
50 55 60

Thr His Phe Val Leu Asn Phe Lys Thr Asn Lys Lys Ser Cys Ile Ser  
65 70 75 80

Ala Ile Lys Leu Thr Ser Tyr Pro Lys Ile Asn Gln Asn Ser Asp Leu  
85 90 95

Thr Lys Asn Leu Tyr Cys Gln Thr Gly Gly Ile Gly Thr Asp Asn Cys  
100 105 110

Lys Leu Val Phe Lys Lys Arg Lys Arg Gln Ile Ala Ala Asn Ile Glu  
115 120 125

Ile Tyr Gly Ile Pro Ala Lys Lys Cys Ser Phe Lys Asp Arg Tyr Ile  
130 135 140

Gly Ala Asp Pro Leu His Val Asp Ser Tyr Gly Leu Pro Tyr Gln Phe  
145 150 155 160

Asp Gln Glu His Gly Trp Asn Val Glu Arg Tyr Asn Ile Phe Lys Asp  
165 170 175

Thr Arg Phe Ser Thr Glu Val Phe Tyr His Lys Asn Gly Leu Phe Asn  
180 185 190

Thr Gln Ile Thr Tyr Leu Ala Glu Glu Asp Ser Phe Ser Glu Ala Arg  
195 200 205

Glu Ile Thr Ala Lys Asp Ile Lys Lys Phe Ser Ile Ile Leu Pro  
210 215 220

Asn Glu Glu Tyr Lys Arg Ile Ser Phe Leu Asp Val Tyr Trp Phe Gln  
225 230 235 240

Glu Thr Met Arg Lys Lys Pro Lys Tyr Pro Tyr Ile His Tyr Asn Gly  
245 250 255

Glu	Cys	Ser	Asn	Glu	Asn	Lys	Thr	Cys	Glu	Leu	Val	Phe	Asp	Thr	Asp
260								265					270		

Glu	Leu	Met	Thr	Tyr	Ala	Leu	Val	Lys	Val	Phe	Thr	Asn	Pro	Glu	Ser
275							280					285			

Asp	Gly	Ser	Arg	Leu	Lys	Glu	Glu	Asp	Leu	Gly	Arg	Gly		
290						295						300		

&lt;210&gt; 16

&lt;211&gt; 1021

&lt;212&gt; DNA

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 16

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gatgaatatt	tcattggaaa	atacaaagaa	aaagatgaga	caactgtttt	tgcaagctac	180
ggcctaaaga	gggatccttg	ccaaattgtc	ttaggctaca	aatgctaaa	caatcaaacc	240
cactttgtgc	ttaattttaa	aaccaataag	aaatcctgca	tatcagcaat	taagctgact	300
tcttacccaa	aaatcaatca	aaactcgat	ttaactaaaa	atctctactg	ccaaactgga	360
ggaataggaa	cagataactg	caaactgtc	ttcaagaaac	gtaaaagaca	aatagcagct	420
aatattgaaa	tctacggcat	tccagcgaag	aaatgttcct	tcaaggatcg	ttacattgga	480
gctgatccac	tccacgtcga	ttcctatggg	cttcgtatc	agtttgcata	ggaacatgga	540
tggaatgtgg	aacgatataa	cattttcaaa	gacacaagat	tttccacaga	agttttctac	600
cacaaaaatg	gtttattttaa	cacccaaata	acttatttgg	ctgaagaaga	ttccttctct	660
gaagctcgag	agattactgc	gaaggatatt	aagaagaagt	tttcaattat	tttgcggaaat	720
gaagagtata	agaggattag	tttcttggac	gtttatttgg	tccaggagac	tatgcgaaaa	780
aagcctaaat	atccctacat	tcactacaat	ggagaatgca	gcaatgagaa	taaaacttgc	840
gaacttgcct	ttgacaccga	tgaactaatg	acctacgccc	ttgttaaagt	ctttactaat	900
cctgagagtg	atggatctag	gctcaaagaa	gaggatttgg	gaagaggata	aatcttctta	960
ataaaaaaaaaa	gttctgttaag	aaaatattgt	tcaataaaatt	aaaaaaaaaa	aaaaaaaaaa	1020
a						1021

&lt;210&gt; 17

&lt;211&gt; 161

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 17

Met	Ala	Phe	Ser	Asn	Thr	Leu	Phe	Val	Leu	Phe	Val	Ser	Phe	Leu	Thr
1						5			10					15	

Phe	Cys	Gly	Ala	Asp	Gln	Thr	Leu	Ile	Glu	Lys	Glu	Leu	Thr	Gly	Arg
							20		25				30		

Thr	Val	Tyr	Ile	Ser	Lys	Ile	Lys	Leu	Asn	Trp	Asn	Asp	Ala	Phe	Asp
						35		40				45			

Tyr	Cys	Ile	Arg	Asn	Gly	Leu	Thr	Phe	Ala	Lys	Ile	Lys	Ser	Ala	Glu
						50		55			60				

Glu	Asn	Thr	Glu	Leu	Ser	Glu	Lys	Leu	Lys	Thr	Val	Ile	Arg	Thr	Glu
						65		70		75			80		

Glu	Phe	Gln	Val	Trp	Ile	Gly	Gly	Ile	Glu	His	His	Gln	Asp	Ser	Ser
						85		90				95			

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

	115	120	125
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Glu	Tyr	Cys	Leu	Glu	Ile	Leu	Phe	Arg	Lys	Glu	Asp	Gly	Lys	Trp	Asn
						130		135			140				

Asp	Phe	Pro	Cys	Ser	Ala	Arg	His	His	Phe	Val	Cys	Glu	Lys	Arg	Thr
						145		150			155			160	

Lys

<210>	18
<211>	586
<212>	DNA
<213>	Lutzomyia longipalpis

<400>	18					
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actgtttata	tctccaaaat	taagctaaat	tggAACgtat	ccttcgatta	ctgcatccgc	180
aatggccctca	cctttgctaa	gattaaatca	gctgaagaaa	acaccgaact	gagtgagaaa	240
ctcaagacag	tcattcgtac	ggaggagttt	caagtttgga	ttggaggcat	tgaacatcat	300
caagacagtt	ccttccgctg	ggtaagcgac	tcccaaccaa	taaccaacaa	attgggctac	360

aaatacacaaa	actggaaatac	cgaggagccc	acaaattacc	aaaacaacga	atattgcttg	420
gaaatattat	tccggaagga	agatggaaaa	tggaatgatt	ttccctgcag	tgcaagacat	480
cattttgttt	gtgaaaaaaag	aacaaaataa	aatgaagaaaa	atgtgatttt	ccttggttg	540
aagaataaaaa	ttctgttgaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaa		586

<210> 19  
<211> 105  
<212> PRT  
<213> Lutzomyia longipalpis

<400> 19

Met Gln Asn Phe Leu Leu Val Ser Leu Ala Leu Ala Ala Leu Met Leu  
1 5 10 15

Cys Ala Glu Ala Lys Pro Tyr Asp Phe Pro Leu Tyr Gln Asp Leu Ile  
20 25 30

Gln Gly Val Ile Gln Arg Glu Ser Gln Ala Glu Arg Glu Lys Arg Ser  
35 40 45

Pro Asn Glu Asp Tyr Glu Lys Gln Phe Gly Asp Ile Val Asp Gln Ile  
50 55 60

Lys Glu Ile Ser Phe Asn Val Met Lys Met Pro His Phe Gly Ser Ser  
65 70 75 80

Asp Asp Asn Arg Asp Asp Gly Glu Tyr Val Asp His His Tyr Gly Asp  
85 90 95

Glu Asp Asp Arg Asp Tyr Asp His Tyr  
100 105

<210> 20  
<211> 457  
<212> DNA  
<213> *Lutzomyia longipalpis*

<400> 20

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gactatgaga agcaatttgg ggatattgtt gatcaaatta aggaaattag tttcaatgtc 240  
atgaaaatgc cccatttgg aagctctgat gataatcgta atgatggcga gtacgttgc 300

catcattatg gtgacgaaga tgatcgat tatgatcatt actaaatact acttgctcct 360  
 gctgaatgac ttgaaggaat catttttg caaaaatatc catcaaatta ttgaattat 420  
 aaagttgcaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaa 457

<210> 21  
 <211> 157  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 21

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

Cys Asn Ala Glu Asp Tyr Asp Lys Val Lys Leu Thr Gly Arg Thr Val  
 20 25 30

Tyr Ile Ser Arg Ser Lys Ala Pro Trp Phe Thr Ala Leu Asp Asn Cys  
 35 40 45

Asn Arg Arg Phe Thr Phe Ala Met Ile Lys Ser Gln Lys Glu Asn Glu  
 50 55 60

Glu Leu Thr Asn Ala Leu Leu Ser Val Ile Lys Ser Asp Glu Glu Asn  
 65 70 75 80

Val Trp Ile Gly Gly Leu Arg His Asp Leu Asp Asp Tyr Phe Arg Trp  
 85 90 95

Ile Ser Phe Gly Thr Ala Leu Ser Lys Thr Ser Tyr Thr Asn Trp Ala  
 100 105 110

Pro Lys Glu Pro Thr Gly Arg Pro His Arg Thr Gln Asn Asp Glu Phe  
 115 120 125

130 135 140

Cys Trp Arg Lys Arg Leu Tyr Val Cys Glu Lys Arg Asp  
 145 150 155

<210> 22  
 <211> 596  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 22  
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 agtaaaaactt actggaagaa ctgtttacat ctccagatca aaggctccgt gttcacagc 180  
 tttagacaat tgtaatcgTT tacgcttcac cttcgccatg atcaagtctc agaaggagaa 240  
 tgaagagcta acaaatgcgc tttaagtgt aattaaatct gacgaagaaa atgtttggat 300  
 tggaggtctt aggcacgatc tggatgacta cttccgttgg attagtttg gaactgcatt 360  
 gtcaaagact tcgtacacca attgggcccc aaaggaaccc acaggaaggc cccatagaac 420  
 tcaaaatgat gaattctgca tgcaaATgtc ttcaaaagat ggtggcaaAT ggagtgataa 480  
 cacctgttgg cgtaaacgtt tgtacgtttg tggaaagcgt gattaaataa aggaacactg 540  
 ccaatgaata ttgggcaatt tgagagaaat taaattaaaa aaaaaaaaaa aaaaaaa 596

&lt;210&gt; 23

&lt;211&gt; 412

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 23

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

His	Gly	Ala	Tyr	Val	Glu	Ile	Gly	Tyr	Ser	Leu	Arg	Asn	Ile	Thr	Phe
					20			25				30			

Asp	Gly	Leu	Asp	Thr	Asp	Asp	Tyr	Asn	Pro	Lys	Phe	Asn	Ile	Pro	Thr
					35		40					45			

Gly	Leu	Ala	Val	Asp	Pro	Glu	Gly	Tyr	Arg	Leu	Phe	Ile	Ala	Ile	Pro
					50		55			60					

Arg	Arg	Lys	Pro	Lys	Val	Pro	Tyr	Thr	Val	Ala	Glu	Leu	Asn	Met	Val
					65		70		75				80		

Met	Asn	Pro	Gly	Phe	Pro	Val	Glu	Arg	Ala	Pro	Ser	Phe	Glu	Lys	Phe
					85		90		95						

Lys	Lys	Phe	Asn	Gly	Glu	Gly	Lys	Lys	Asp	Leu	Val	Asn	Val	Tyr	Gln
					100		105					110			

Pro	Val	Ile	Asp	Asp	Cys	Arg	Arg	Leu	Trp	Val	Leu	Asp	Ile	Gly	Lys
					115		120				125				

Val	Glu	Tyr	Thr	Gly	Gly	Asp	Ala	Asp	Gln	Tyr	Pro	Lys	Gly	Lys	Pro
					130		135		140						

Thr Leu Ile Ala Tyr Asp Leu Lys Lys Asp His Thr Pro Glu Ile His  
145 150 155 160

Arg Phe Glu Ile Pro Asp Asp Leu Tyr Ser Ser Gln Val Glu Phe Gly  
165 170 175

Gly Phe Ala Val Asp Val Val Asn Thr Lys Gly Asp Cys Thr Glu Ser  
180 185 190

Phe Val Tyr Leu Thr Asn Phe Lys Asp Asn Ser Leu Ile Val Tyr Asp  
195 200 205

Glu Thr Gln Lys Lys Ala Trp Lys Phe Thr Asp Lys Thr Phe Glu Ala  
210 215 220

225 230 235 240

Lys Val Gly Leu Phe Gly Ile Ala Leu Gly Asp Arg Asp Glu Met Gly  
245 250 255

His Arg Pro Ala Cys Tyr Ile Ala Gly Ser Ser Thr Lys Val Tyr Ser  
260 265 270

Val Asn Thr Lys Glu Leu Lys Thr Glu Asn Gly Gln Leu Asn Pro Gln  
275 280 285

Leu His Gly Asp Arg Gly Lys Tyr Thr Asp Ala Ile Ala Leu Ala Tyr  
290 295 300

Asp Pro Glu His Lys Val Leu Tyr Phe Ala Glu Ser Asp Ser Arg Gln  
305 310 315 320

Val Ser Cys Trp Asn Val Asn Met Glu Leu Lys Pro Asp Asn Thr Asp  
325 330 335

Val Ile Phe Ser Ser Ala Arg Phe Thr Phe Gly Thr Asp Ile Leu Val  
340 345 350

Asp Ser Lys Gly Met Leu Trp Ile Met Ala Asn Gly His Pro Pro Val  
355 360 365

Glu Asp Gln Glu Lys Ile Trp Lys Met Arg Phe Val Asn Arg Lys Ile  
370 375 380

Arg Ile Met Lys Val Asp Thr Glu Arg Val Phe Lys Tyr Ser Arg Cys

385

390

395

400

Asn Pro Asn Tyr Lys Pro Pro Lys Glu Ile Glu Val  
 405 410

<210> 24  
 <211> 1409  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 24  
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 attcgatgga ttggatacag atgactacaa tccaaagttc aacattccaa cgggtttggc 180  
 agttgatccc gaaggatata ggctcttcat agccatccca aggagaaagc caaaggttcc 240  
 ctacactgtg gctgaactga atatggtcat gaatcccgga tttccgtcg agagagctcc 300  
 gagcttttag aaattcaaaa aattcaatgg cgagggcaaa aaggatcttgc ttaatgtgt 360  
 tcagccagtc attgatgatt gtcgtcgtct ttgggtgctt gacattggga aggtggaata 420  
 caccgggtgtt gatgctgatc aatatccaa agaaaagcct accctaattt cctacgaccc 480  
 caagaaggat catactccgg aaattcatcg atttgaattt ccagacgatc tctatacgctc 540  
 acaagttgaa ttgggtggat ttgccgttga tgggttaac acgaaaggag actgtacgg 600  
 gtcatttgct tacctgacca atttcaagga taactctcta attgtctacg atgagacaca 660  
 aaagaaaact tggaaattca cagataaaac atttgaagct gataaggaat ccacgttctc 720  
 ctactcggga gaggaacaaa tgaagtacaa agtcggtctt ttggatag ctctgggtga 780  
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 cagtgttaac actaaagaac tcaaaacaga gaatggtcag ttaaatcctc agttcacgg 900  
 tgatcgtgga aagtacacag atgcaattgc cctagcctac gatcctgagc ataaagtctc 960  
 ctactttgct gaatccgaca gcaggcaggt gtcctgttgg aatgtaaata tggagctaaa 1020  
 accagacaat acggatgtga tttctcttag tgcccgaaaa actttggaa cggatatttt 1080  
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 ggaacgtgtt ttcaaatatt cacgctgcaaa tccaaattat aagcccccaa agggaaattgt 1260  
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 aaaaaaaaaa aaaaaaaaaa aaaaaaaaa 1409

<210> 25  
<211> 295  
<212> PRT  
<213> Lutzomyia longipalpis

<400> 25

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

Cys Ala Asn Glu Ile Pro Ile Asn Arg Gln Gly Lys Asp Tyr Pro Val  
20 25 30

Pro Ile Ile Asp Pro Asn Lys Ser Ser Ser Asp Asp Tyr Phe Asp Asp  
35 40 45

Arg Phe Tyr Pro Asp Ile Asp Asp Glu Gly Ile Ala Glu Ala Pro Lys  
50 55 60

Asp Asn Arg Gly Lys Ser Arg Gly Gly Ala Ala Gly Ala Arg Glu  
65 70 75 80

Gly Arg Leu Gly Thr Asn Gly Ala Lys Pro Gly Gln Gly Gly Thr Arg  
85 90 95

Pro Gly Gln Gly Gly Thr Arg Pro Gly Gln Gly Gly Thr Arg Pro Gly  
100 105 110

Gln Gly Gly Thr Arg Pro Gly Gln Gly Gly Thr Arg Pro Gly Gln Gly  
115 120 125

Arg Thr Lys Pro Ala Gln Gly Thr Thr Arg Pro Ala Gln Gly Thr Arg  
130 135 140

Asn Pro Gly Ser Val Gly Thr Lys Glu Ala Gln Asp Ala Ser Lys Gln  
145 150 155 160

Gly Gln Gly Lys Arg Arg Pro Gly Gln Val Gly Gly Lys Arg Pro Gly  
165 170 175

Gln Ala Asn Ala Pro Asn Ala Gly Thr Arg Lys Gln Gln Lys Gly Ser  
180 185 190

Arg Gly Val Gly Arg Pro Asp Leu Ser Arg Tyr Lys Asp Ala Pro Ala  
195 200 205

Lys Phe Val Phe Lys Ser Pro Asp Phe Ser Gly Glu Gly Lys Thr Pro

210	215	220
Thr Val Asn Tyr Phe Arg Thr Lys Lys Lys Glu His Ile Val Thr Arg		
225	230	235
240		
Gly Ser Pro Asn Asp Glu Phe Val Leu Glu Ile Leu Asp Gly Asp Pro		
245	250	255
Thr Gly Leu Gly Leu Lys Ser Glu Thr Ile Gly Lys Asp Thr Arg Leu		
260	265	270
Val Leu Glu Asn Pro Asn Gly Asn Ser Ile Val Ala Arg Val Lys Ile		
275	280	285
Tyr Lys Asn Gly Tyr Ser Gly		
290	295	
<210> 26		
<211> 989		
<212> DNA		
<213> Lutzomyia longipalpis		
<400> 26		
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cagttccgat cattgatcca aataaatcat cttcggatga ttatttcgat gatcgcttct 180		
accctgatat tcatgtatgag ggcatacgctg aggctcctaa ggataatagg ggaaaatccc 240		
gtgggtgtgg tgcggctggc gcaagagaag gttaggttagg tacgaatggg gctaaaccgg 300		
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agcctgctca ggaaactact aggccagctc agggaaactag aaatccagga tcggttggta 480		
cgaaagaagc ccaggatgctg tcaaaaacaag gtcaaggtaa aagaaggcca gggcaagttg 540		
gtggtaaaag accaggacaa gcaaattgctc ctaatgcagg cactagaaag caacagaaaag 600		
gcagtagagg cgttggaaagg cctgatctat cgcgctacaa agatccccct gctaaattcg 660		
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acggttattc aggtgaaga agaaatcctt tgattcccc ccccccctct tcctttaaaa 960		

ttcaacataa taaaaaaaaaaa aaaaaaaaaa 989

<210> 27  
 <211> 148  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 27

Met Asn Ser Val Asn Thr Leu Ile Leu Thr Leu Leu Phe Ala Ile Phe  
 1 5 10 15

Leu Leu Val Lys Arg Ser Gln Ala Phe Leu Pro Ser Asp Pro Ser Ile  
 20 25 30

Cys Val Lys Asn Leu Val Leu Asp Thr Gly Arg Thr Cys Glu Glu Ser  
 35 40 45

Glu Tyr Phe Pro Asp Ile Lys Asn Val Lys Asn Gly Lys Arg Val Tyr  
 50 55 60

Ile Val Cys Thr Asp Ser Asp Ala Val Asp Tyr Lys Phe Tyr Ile Cys  
 65 70 75 80

Phe Asp Met Asn Arg Leu Ser Gly Pro Pro Tyr Pro Glu Glu Ile  
 85 90 95

Leu Arg Glu Ser Thr Val Thr Tyr Ala Gln Ile Tyr Glu Leu Met Thr  
 100 105 110

Thr Glu Thr Glu Thr Lys Lys Pro Lys Lys Pro Lys Asn Ser  
 115 120 125

Lys Thr Asp Asp Pro Pro Ala Ile Arg Pro Gly Phe Ser Phe Arg Asn  
 130 135 140

Ser Ile Ser Val  
 145

<210> 28  
 <211> 826  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 28

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 tctatggca attttttat tagtgaaaag gtctcaggct tttcttccat ctgaccgaag 120  
 tatctgtgtt aaaaattttag tattggatac aggaaggact tgtgaggaaa gtgaatattt 180

tccggatatac aagaacgtta	aaaatggaaa aagagttac	attgtctgca	ctgattcaga	240
tgcagttgat tataaaatttt	atatttgttt cgatatgaat	cgtcttctg	gaccaccgta	300
tcctgaggaa gaaaatccttc	gtgaatcaac ggtaacttat	gcccaaattt	atgagctgat	360
gactacggaa accactgaaa	ccaaaaagcc aaaaaagaaa	ccaaagaatt	caaaaacgga	420
cccagaccct ccagcaattc	gtccaggatt ttcattttaga	aattcaattt	ctgtttaatt	480
ttacaattta ttttggaaaga	aaaatgatat ttcgaaatat	tctatacataaa	aaaacaacag	540
ttataaaaacg aaaattcaat	catttcaatg agaaaactta	gtcttgagta	aggtttattc	600
accacccgac gccacgctat	ggtgaataat ttctttatt	caccacatca	aaatgacggc	660
ttataaaactt caacaaatag	tttggaaaat acatttctaa	ctaatgcaat	gtttacttaa	720
aatcaattta caaattcacg	catttgagat gcaacaaata	tatacaattc	aacgatataaa	780
actttccaca agaaaaactt	tcaacccaaa aaaaaaaaaa	aaaaaaa	aaaaaa	826

<210> 29

<211> 397

<212> PRT

<213> Lutzomyia longipalpis

<400> 29

Met	Lys	Leu	Phe	Phe	Phe	Leu	Tyr	Thr	Phe	Gly	Leu	Val	Gln	Thr	Ile
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Phe	Gly	Val	Glu	Ile	Lys	Gln	Gly	Phe	Lys	Trp	Asn	Lys	Ile	Leu	Tyr
			20					25				30			

Glu	Gly	Asp	Thr	Ser	Glu	Asn	Phe	Asn	Pro	Asp	Asn	Asn	Ile	Leu	Thr
					35			40				45			

Ala	Phe	Ala	Tyr	Asp	Pro	Glu	Ser	Gln	Lys	Leu	Phe	Leu	Thr	Val	Pro
					50			55			60				

Arg	Lys	Tyr	Pro	Glu	Thr	Met	Tyr	Thr	Leu	Ala	Glu	Val	Asp	Thr	Glu
					65			70			75		80		

Lys	Asn	Ser	Phe	Glu	Ser	Gly	Asp	Thr	Ser	Pro	Leu	Leu	Gly	Lys	Phe
					85			90				95			

Ser	Gly	His	Glu	Thr	Gly	Lys	Glu	Leu	Thr	Ser	Val	Tyr	Gln	Pro	Val
					100			105			110				

Ile	Asp	Glu	Cys	His	Arg	Leu	Trp	Val	Val	Asp	Val	Gly	Ser	Val	Glu
					115			120			125				

Arg Asn Ser Asp Gly Thr Glu Gly Gln Pro Glu His Asn Pro Thr Leu  
130 135 140

Val Ala Tyr Asp Leu Lys Glu Ala Asn Tyr Pro Glu Val Ile Arg Tyr  
145 150 155 160

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

Ala Val Asp Val Val Lys Pro Asp Glu Cys Ser Glu Thr Phe Val Tyr  
180 185 190

Ile Thr Asn Phe Leu Thr Asn Ala Leu Ile Val Tyr Asp His Lys Asn  
195 200 205

Lys Asp Ser Trp Thr Val Gln Asp Ser Thr Phe Gly Pro Asp Lys Lys  
210 215 220

Ser Lys Phe Asp His Asp Gly Gln Gln Tyr Glu Tyr Glu Ala Gly Ile  
225 230 235 240

Phe Gly Ile Thr Leu Gly Glu Arg Asp Asn Glu Gly Asn Arg Gln Ala  
245 250 255

Tyr Tyr Leu Val Ala Ser Ser Thr Lys Leu His Ser Ile Asn Thr Lys  
260 265 270

Glu Leu Lys Gln Lys Gly Ser Lys Val Asn Ala Asn Tyr Leu Gly Asp  
275 280 285

Arg Gly Glu Ser Thr Asp Ala Ile Gly Leu Val Tyr Asp Pro Lys Thr  
290 295 300

Lys Thr Ile Phe Phe Val Glu Ser Asn Ser Lys Arg Val Ser Cys Trp  
305 310 315 320

Asn Thr Gln Glu Thr Leu Asn Lys Asp Lys Ile Asp Val Ile Tyr His  
325 330 335

Asn Ala Asp Phe Ser Phe Gly Thr Asp Ile Ser Ile Asp Ser Gln Asp  
340 345 350

Asn Leu Trp Phe Leu Ala Asn Gly Leu Pro Pro Leu Glu Asn Ser Asp  
355 360 365

Lys Phe Val Phe Thr Lys Pro Arg Tyr Gln Ile Phe Lys Val Asn Ile  
 370 375 380

Gln Glu Ala Ile Ala Gly Thr Lys Cys Glu Lys Asn Leu  
 385 390 395

<210> 30  
 <211> 1325  
 <212> DNA  
 <213> *Lutzomyia longipalpis*

<400> 30  
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 agggcgatac atcagaaaac ttcaatccag ataacaacat cttacggct tttgcgtacg 180  
 atcctgagag tcagaaaactc ttccctaactg tcccggagaa atatccgaa actatgtaca 240  
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 ttggaaaatt cagtggtcat gaaactggga aagaacttac atcagttat cagccagtt 360  
 tcgatgaatg tcatcgctt tgggttggatc atgttggatc agtagaacgt aactcagacg 420  
 gcacagaagg tcagccagaa cataatccta cccttggc gtacgatctc aaagaagcca 480  
 actatcctga agttattcgt tacacgttcc cgataattc cattgagaag cccacatttc 540  
 tgggtggatt tgccgttgat gttgtaaagc cgatgaaatc cagtggaaact tttgtctaca 600  
 tcacaaaactt cctcaccaac gccctcatag tatacgatca taagaataag gactcctgga 660  
  
 agtatgaata cgaagcagga atcttcggga ttacccttgg agagaagat aacgaaggaa 780  
 atcgtcaagc gtactattta gtagcaagta gtaccaaact tcacagcatc aacaccaaag 840  
 aactgaagca aaaaggaagc aaagttaatg caaatttattt gggagatcgt ggtgaatcca 900  
 ccgatgccat aggcttagtt tacgatccaa aaaccaaaac tatcttcttc gttgagtcaa 960  
 atagcaaaag agtatcatgc tggaaatcccc agaaacact aaacaaggat aaaattgtat 1020  
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 atttgggtt cctagcaaat ggacttccac ctctggaaaa ttctgataaa tttgtcttta 1140  
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 aaaaaa 1325

<210> 31  
 <211> 350  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 31

Met Thr Phe Leu Ile Ile Leu Gly Ala Phe Leu Leu Val Gln Ile Ile  
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Thr Ala Ser Ala Leu Gly Leu Pro Glu Gln Phe Lys Gly Leu Glu Asp  
 20 25 30

Leu Pro Lys Lys Pro Leu Ala Glu Thr Tyr Tyr His Glu Gly Leu Asn  
 35 40 45

Asp Gly Lys Thr Asp Glu Met Val Asp Ile Phe Lys Ser Leu Ser Asp  
 50 55 60

Glu Phe Lys Phe Ser Asp Glu Asn Leu Asp Val Gly Glu Glu Lys Asn  
 65 70 75 80

Tyr Lys Lys Arg Asp Ile Thr Gln Asn Ser Val Ala Arg Asn Phe Leu  
 85 90 95

Ser Asn Val Lys Gly Ile Pro Ser Met Pro Ser Leu Pro Ser Met Pro  
 100 105 110

Ser Met Pro Ser Ile Pro Ser Leu Trp Ser Ser Gln Thr Gln Ala Ala  
 115 120 125

Pro Asn Thr Ala Leu Ala Leu Pro Glu Ser Asp Tyr Ser Leu Leu Asp  
 130 135 140

Met Pro Asn Ile Val Lys Asn Phe Leu Lys Glu Thr Arg Asp Leu Tyr  
 145 150 155 160

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

Arg Ser Ser Ser Ser Gln Leu Leu Ser Ser Pro Met Val Ser Thr Asn  
 180 185 190

Lys Thr Lys Glu Phe Ile Arg Asn Glu Ile Gln Lys Val Arg Lys Val  
 195 200 205

Arg Asn Phe Val Gln Glu Thr Leu Gln Lys Ile Arg Asp Ile Ser Ala  
 210 215 220

Ala Ile Ala Lys Lys Val Lys Ser Ser Glu Cys Leu Ser Asn Leu Thr  
 225 230 235 240

Asp Ile Lys Gly Leu Val Ser Asp Gly Ile Asn Cys Leu Lys Glu Lys  
 245 250 255

Phe Asn Asp Gly Lys Arg Ile Ile Leu Gln Leu Tyr Asn Asn Leu Leu  
 260 265 270

Lys Gly Leu Lys Ile Pro Asn Asp Leu Met Val Glu Leu Lys Lys Cys  
 275 280 285

Asp Thr Asn Gln Asn Asn Thr Leu Gly Arg Ile Ile Cys Tyr Phe Leu  
 290 295 300

Thr Pro Leu Gln Leu Glu Lys Glu Gln Ile Leu Leu Pro Val Glu Phe  
 305 310 315 320

Ile Lys Arg Ile Leu Glu Leu Thr His Tyr Phe Ser Thr Met Lys Glu  
 325 330 335

Asp Leu Ile Asn Cys Gly Ile Thr Thr Ile Ala Ser Ile Thr  
 340 345 350

<210> 32  
 <211> 1275  
 <212> DNA  
 <213> Lutzomyia longipalpis

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 ttaaaggattt agaggattt cctaaaaaac cttggcaga gacttattat cacgaaggat 180  
 tgaatgatgg aaaaacggat gaaatggtgg atattttaa aagtcttagc gatgaattt 240  
 aattcagtga tgaaaatttta gatgttggtg aggagaaaa ttacaagaaa cgtgatataa 300  
 cccaaaattt cgtggcaagg aacttcctat caaacgtaaa gggatttcct tcaatgccat 360  
 cactcccttc aatgccttca atgccatcaa ttcccttact ttggtcaagt cagacacagg 420  
 cggcaccaaa taccgcactt gcccttcctg aatctgatta ttcccttcta gatatgccga 480  
 atattgtgaa aaatttccta aaggaaacaa gagacctcta taacgatgtt ggagctttc 540  
 ttaaggcaat tacagaagct ttaacaaata gatcttcatc atctcaactt ctttcctccc 600  
 caatggtgag cacgaataaa accaaagaat ttattcggaa tgaaatacaa aaagtccgaa 660

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ccaaaaaggt	aaaatcatca	gaatgtctgt	ccaatcttac	ggacatcaaa	ggacttgtat	780
cagacggaat	taattgttta	aaggaaaaat	tcaatgatgg	aaaacgaatt	atcctgcaat	840
tgtacaataa	tttactaaaa	ggactcaaaa	ttccaaatga	cctaatggtt	gaattgaaga	900
aatgtgatac	aaatcaaaac	aatactttgg	gaagaataat	ctgttatttt	ttgacaccat	960
tgcaactgga	aaaagaacaa	attcttctac	ctgtagaatt	tataaagcgc	attcttgaat	1020
taacccacta	ctttccaca	atgaaagaag	atcttatcaa	ctgtggcatc	acaacgattg	1080
catccattac	gtaaaaaatg	gaaaaatgtg	ccggtgaaat	gcttgaatc	accaaagaaa	1140
tttcatcgca	aataacagtt	ccagaataac	caaattttaa	tgattacttc	tcaaggaaaa	1200
tactaccaaa	aggcattaat	taaaacgatg	tttttataaa	acaatgtaag	aaaaaaaaaa	1260
aaaaaaaaaa	aaaaaa					1275

&lt;210&gt; 33

&lt;211&gt; 60

&lt;212&gt; PRT

<213> *Lutzomyia longipalpis*

&lt;400&gt; 33

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

Cys	Val	Ala	Ala	Met	Pro	Gly	Ser	Asn	Val	Pro	Trp	His	Ile	Ser	Arg
				20				25				30			

Glu	Glu	Leu	Glu	Lys	Leu	Arg	Glu	Ala	Arg	Lys	Asn	His	Lys	Ala	Leu
				35			40			45					

Glu	Lys	Ala	Ile	Asp	Glu	Leu	Ile	Asp	Lys	Tyr	Leu
				50		55		60			

&lt;210&gt; 34

&lt;211&gt; 413

&lt;212&gt; DNA

<213> *Lutzomyia longipalpis*

&lt;400&gt; 34

agttaatctt	ctgtcaagct	acaaaaatgc	ttaaaatcgt	tttatttcta	tcagttttgg	60
ctgtatttagt	gatttgtgta	gcagcaatgc	caggatccaa	tgttccttgg	cacatttcac	120
gagaagagct	tgagaagctt	cgtgaagctc	gaaagaatca	caaggcactc	gagaaggcaa	180
ttgatgaatt	aattgacaaa	tatctctgat	tttgaagagc	aaggaagagg	aaataaacgg	240

ccgaggaagg attttcttta gagattcttc tttttattac ttcaaacccta acttcaaaat 300  
 cagtcgtata ttttttaat ttgaaaaaaaaa tattgaaaat tttaactatt tgtgaaattt 360  
 aaataaataa agaatgtcag aagcaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 413

<210> 35  
 <211> 120  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 35

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

Phe Tyr Arg Val Glu Gly Ser Ser Gln Cys Glu Glu Asp Leu Lys Glu  
 20 25 30

Glu Ala Glu Ala Phe Phe Lys Asp Cys Asn Glu Ala Lys Ala Asn Pro  
 35 40 45

Gly Glu Tyr Glu Asn Leu Thr Lys Glu Glu Met Phe Glu Glu Leu Lys  
 50 55 60

Glu Tyr Gly Val Ala Asp Thr Asp Met Glu Thr Val Tyr Lys Leu Val  
 65 70 75 80

Glu Glu Cys Trp Asn Glu Leu Thr Thr Asp Cys Lys Arg Phe Leu  
 85 90 95

Glu Glu Ala Glu Cys Phe Lys Lys Asn Ile Cys Lys Tyr Phe Pro  
 100 105 110

Asp Glu Val Lys Leu Lys Lys  
 115 120

<210> 36  
 <211> 428  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 36  
 aattttcacc atgaagtttt cttgccagt tttcggtgca attttccttt tgtgcggatt 60  
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 ctttaaggat tgcaatgaag caaaagccaa tcctggtgaa tacgagaatc tcaccaaaga 180  
 agaaatgtt gaagaattga aagaatatgg agttgctgac acagacatgg agacagttt 240  
 caaacttgtg gaagaatgtt ggaatgaatt aacaacaacg gattgtaaga gatttctcga 300

agaggctgaa tgcttcaaga agaagaatat ttgtaaatat ttcccagatg aagtgaatt 360  
 gaagaagaaa taaatttta gcttggaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 420  
 aaaaaaaaaa 428

<210> 37  
 <211> 572  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 37

Met Leu Phe Phe Leu Asn Phe Phe Val Leu Val Phe Ser Ile Glu Leu  
 1 5 10 15

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

Ile Ile Leu His Thr Asn Asp Met His Ala Arg Phe Asp Gln Thr Asn  
 35 40 45

Ala Gly Ser Asn Lys Cys Gln Glu Lys Asp Lys Ile Ala Ser Lys Cys  
 50 55 60

Tyr Gly Gly Phe Ala Arg Val Ser Thr Met Val Lys Lys Phe Arg Glu  
 65 70 75 80

Glu Asn Gly Ser Ser Val Leu Phe Leu Asn Ala Gly Asp Thr Tyr Thr  
 85 90 95

Gly Thr Pro Trp Phe Thr Leu Tyr Lys Glu Thr Ile Ala Thr Glu Met  
 100 105 110

Met Asn Ile Leu Arg Pro Asp Ala Ala Ser Leu Gly Asn His Glu Phe  
 115 120 125

Asp Lys Gly Val Glu Gly Leu Val Pro Phe Leu Asn Gly Val Thr Phe  
 130 135 140

Pro Ile Leu Thr Ala Asn Leu Asp Thr Ser Gln Glu Pro Thr Met Thr  
 145 150 155 160

Asn Ala Lys Asn Leu Lys Arg Ser Met Ile Phe Thr Val Ser Gly His  
 165 170 175

Arg Val Gly Val Ile Gly Tyr Leu Thr Pro Asp Thr Lys Phe Leu Ser  
 180 185 190

Asp Val Gly Lys Val Asn Phe Ile Pro Glu Val Glu Ala Ile Asn Thr  
195 200 205

Glu Ala Gln Arg Leu Lys Lys Glu Glu Asn Ala Glu Ile Ile Ile Val  
210 215 220

Val Gly His Ser Gly Leu Ile Lys Asp Arg Glu Ile Ala Glu Lys Cys  
225 230 235 240

Pro Leu Val Asp Ile Ile Val Gly Gly His Ser His Thr Phe Leu Tyr  
245 250 255

Thr Gly Ser Gln Pro Asp Arg Glu Val Pro Val Asp Val Tyr Pro Val  
260 265 270

Val Val Thr Gln Ser Ser Gly Lys Lys Val Pro Ile Val Gln Ala Tyr  
275 280 285

Cys Phe Thr Lys Tyr Leu Gly Tyr Phe Lys Val Thr Ile Asn Gly Lys  
290 295 300

Gly Asn Val Val Gly Trp Thr Gly Gln Pro Ile Leu Leu Asn Asn Asn  
305 310 315 320

Ile Pro Gln Asp Gln Glu Val Leu Thr Ala Leu Glu Lys Tyr Arg Glu  
325 330 335

Arg Val Glu Asn Tyr Gly Asn Arg Val Ile Gly Val Ser Arg Val Ile  
340 345 350

Leu Asn Gly Gly His Thr Glu Cys Arg Phe His Glu Cys Asn Met Gly  
355 360 365

Asn Leu Ile Thr Asp Ala Phe Val Tyr Ala Asn Val Ile Ser Thr Pro  
370 375 380

Met Ser Thr Asn Ala Trp Thr Asp Ala Ser Val Val Leu Tyr Gln Ser  
385 390 395 400

Gly Gly Ile Arg Ala Pro Ile Asp Pro Arg Thr Ala Ala Gly Ser Ile  
405 410 415

Thr Arg Leu Glu Leu Asp Asn Val Leu Pro Phe Gly Asn Ala Leu Tyr  
420 425 430

Val Val Lys Val Pro Gly Asn Val Leu Arg Lys Ala Leu Glu His Ser  
 435 440 445

Val His Arg Tyr Ser Asn Thr Ser Gly Trp Gly Glu Phe Pro Gln Val  
 450 455 460

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

Val Lys Ser Val Lys Val Leu Cys Ser Asn Cys Ser Gln Pro Glu Tyr  
 485 490 495

Gln Pro Leu Arg Asn Lys Lys Thr Tyr Asn Val Ile Met Asp Ser Phe  
 500 505 510

Met Lys Asp Gly Gly Asp Gly Tyr Ser Met Phe Lys Pro Leu Lys Ile  
 515 520 525

Ile Lys Thr Leu Pro Leu Gly Asp Ile Glu Thr Val Glu Ala Tyr Ile  
 530 535 540

Glu Lys Met Gly Pro Ile Phe Pro Ala Val Glu Gly Arg Ile Thr Val  
 545 550 555 560

Leu Gly Gly Leu Gln Lys Ser Asp Glu Asp Trp His  
 565 570

<210> 38

<211> 1839

<212> DNA

<213> Lutzomyia longipalpis

<400> 38

agttgcaaga	atttcttcat	tgcgttaaga	tgttgttttt	ccttaacttt	tttgtgctgg	60
tgttcagcat	agaactggcg	ttgttaacag	catcagcagc	agcagaagac	ggcagctatg	120
agatcataat	tcttcacacc	aatgatatgc	acgcgcgttt	tgatcaaacc	aatgctggaa	180
gcaacaaatg	ccaagaaaaaa	gacaagattg	cttccaaatg	ctacggagga	tttgcagag	240
tttcaacaat	ggtgaaaaaa	ttccgagaag	aaaatggcag	cagtgtcttg	ttcttgaatg	300
ctggtgacac	gtatacaggt	accccatggt	ttaccctcta	caaggagacc	attgcaacgg	360
agatgatgaa	catccttcgt	ccagatgcag	cctcaactggg	aaatcatgaa	ttcgacaaag	420
gagtagaagg	actcgtgcca	ttcctcaatg	gtgtcacctt	ccctatttta	acagcgaatt	480
tggacacttc	tcaagagcca	acaatgacca	atgctaaaaaa	tctcaaacgc	tcaatgattt	540
ttacggttcc	cgggcacaga	gttgggttaa	ttggctacct	aacgcctgat	acaaaattcc	600

tctcgacgt	tggtttaagtt	aattttattc	cggaagttga	agccatcaat	acggaagcac	660
agcgtctgaa	gaaagaggaa	aatgccaaa	taatcatcgt	tgttggacat	tcagggttga	720
taaaagatcg	agaaattgca	gagaaatgcc	cactggttga	cataattgtt	ggaggacatt	780
cacacacatt	cctctacaca	ggaagtcagc	ctgatcgtga	ggttcctgta	gacgtttata	840
ctgttgttgt	gacccaatcc	agtggaaaga	aagttccaat	tgttcaagcc	tattgcttta	900
caaagtattt	ggggtacttt	aaagtgacga	tcaacggaaa	aggaaatgtt	gtgggatgga	960
ctgggcagcc	aattctcctt	aataacaaca	ttccccaaaga	tcaagttttttt	ctcaactgctc	1020
ttgaaaagta	cagagaacgc	gtggaaaact	atggaaatcg	cgttcccggt	gtttcccggt	1080
taattctcaa	tggggggcat	actgaatgtc	gtttccatga	atgcaatatg	ggtaatctca	1140
tcacggacgc	ttttgtgtat	gccaatgtaa	tcagtacacc	aatgagtacg	aatgcctgga	1200
cagatgcaag	tgttgttctg	tatcaaagtg	gtggcattcg	tgcccaattt	gatcctcgta	1260
ccgcggcagg	gagcatcaca	cgcctcgagt	tggacaatgt	tctaccattt	ggaaatgcac	1320
tgtacgtcgt	aaaagttcct	ggaaatgtct	tacgcaaaggc	tttggaaacat	tcaagtttcatt	1380
gatactccaa	cacttcggga	tggggagaat	ttccacaatgt	ttcggggcta	aagattcggt	1440
ttaacgtcaa	tgaagaaattt	ggaaaacgcg	taaagtccgt	taaagttctc	ttagcaattt	1500
gctctcaacc	tgaataccaa	ccactgagaa	ataaaaaaaac	ttacaacgtt	atcatggaca	1560
gttttatgaa	ggatggaggt	gatgggtata	gcatgttcaa	gcccttgaag	atcatcaaga	1620
ccctccact	gggagatattt	gaaacagtag	aagcttataat	tgagaaaaatg	ggccccattt	1680
tcccagcagt	cgagggaaagg	atcaactgttc	ttggggact	tcaaaaatca	gatgaggatt	1740
ggcattagaa	acatcctgga	cgttatggaa	agaataaaaag	aaggatcata	gaaaaaaaaaa	1800
aaaaaaaaat	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	1839

&lt;210&gt; 39

&lt;211&gt; 86

&lt;212&gt; PRT

<213> *Lutzomyia longipalpis*

&lt;400&gt; 39

Met	Lys	Gln	Ile	Leu	Leu	Ile	Ser	Leu	Val	Val	Ile	Leu	Ala	Val	Leu
1				5					10					15	

Ala	Phe	Asn	Val	Ala	Glu	Gly	Cys	Asp	Ala	Thr	Cys	Gln	Phe	Arg	Lys
				20			25					30			

Ala	Ile	Glu	Asp	Cys	Lys	Lys	Ala	Asp	Asn	Ser	Asp	Val	Leu	Gln
				35			40					45		

Thr Ser Val Gln Thr Thr Ala Thr Phe Thr Ser Met Asp Thr Ser Gln  
 50 55 60

Leu Pro Gly Asn Asn Val Phe Lys Ala Cys Met Lys Glu Lys Ala Lys  
 65 70 75 80

Glu Phe Arg Ala Gly Lys  
 85

<210> 40  
 <211> 419  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 40  
 gtcagtgatc tgataaggta ttaaaatgaa gcaaattcctt ctaatctctt tggtggtgat 60  
 tcttgccgtg cttgccttca atgttgctga gggctgtgat gcaacatgcc aatttcgcaa 120  
 agccatagaa gactgcaaga agaaggcggtaaatagcgat gttttgcaga cttctgtaca 180  
 aacaactgca acattcacat caatggatac atcccaacta cctggaaata atgtcttcaa 240  
 agcatgcattg aaggagaagg ctaaggaatt tagggcagga aagtaagaga ttgaggaaaa 300  
 ttgttagccga agagagaagg aaggaaagtc ccatattttt tttgttaatt gtaacgaatt 360  
 ttgcgaaaaaa aataaaatat tatgcactcc aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 419

<210> 41  
 <211> 84  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 41

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

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

Phe Gln Lys Cys Leu Glu Gln Tyr Pro Glu Pro Asn Gln Ser Gly Glu  
 35 40 45

Val Leu Ala Cys Leu Lys Lys Arg Glu Gly Ala Lys Asp Phe Arg Glu  
 50 55 60

Lys Arg Ser Leu Asp Asp Ile Glu Gly Thr Phe Gln Glu Ser Gly Asn  
 65 70 75 80

Leu Trp Gly Ala

<210> 42  
 <211> 429  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 42  
 tattttaat aattctgtgt aaaatgaacg ttctttcgt gtcttcacg ctcacaattc 60  
 ttcttctctg tgttaaggca cggccagaag atttcgtagc tcttcaggat caagctaatt 120  
 tccagaaatg cctcgaacaa tatccagaac caaatcaatc tggagaagtt cttgcgtgcc 180  
 tcaagaagcg cgaaggtgcc aaagattcc gggaaaagag gagcctggat gacatagaag 240  
 ggactttcca agagtctgga aatctctggg gtgcataggg agtcagagg acttctaatc 300  
 aatctgtgag aagagaaccc aacggctaga gaaaatttaa gggaaaataaa gaaattaatg 360  
 aagcattaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 420  
 aaaaaaaaaa 429

<210> 43  
 <211> 626  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 43

Met	Lys	Ile	Thr	Val	Ile	Leu	Phe	Thr	Gly	Phe	Thr	Ile	Ala	Leu	Val
1				5				10				15			

Ser Ser Ala Val Leu Lys Lys Asn Gly Glu Thr Ile Glu Glu Glu Glu  
 . 20 . 25 . 30

Val Arg Ala Glu Gln Arg Leu Arg Glu Ile Asn Glu Glu Leu Asp Arg  
 35 40 45

Arg Lys Asn Ile Asn Thr Val Ala Ala Trp Ala Tyr Ala Ser Asn Ile  
 50 55 60

Thr Glu Val Asn Leu Lys Asn Met Asn Asp Val Ser Val Glu Thr Ala  
 65 70 75 80

Lys Tyr Tyr Lys Glu Leu Ala Ser Glu Leu Lys Gly Phe Asn Ala Lys  
 85 90 95

Glu Tyr Lys Ser Glu Asp Leu Lys Arg Gln Ile Lys Lys Leu Ser Lys  
 100 105 110

Leu Gly Tyr Ser Ala Leu Pro Ser Glu Lys Tyr Lys Glu Leu Leu Glu  
115 120 125

Ala Ile Thr Trp Met Glu Ser Asn Tyr Ala Lys Val Lys Val Cys Ser  
130 135 140

Tyr Lys Asp Pro Lys Lys Cys Asp Leu Ala Leu Glu Pro Glu Ile Thr  
145 150 155 160

Glu Ile Leu Ile Lys Ser Arg Asp Pro Glu Glu Leu Lys Tyr Tyr Trp  
165 170 175

Lys Gln Trp Tyr Asp Lys Ala Gly Thr Pro Thr Arg Glu Ser Phe Asn  
180 185 190

Lys Tyr Val Gln Leu Asn Arg Glu Ala Ala Lys Leu Asp Gly Phe Tyr  
195 200 205

Ser Gly Ala Glu Ser Trp Leu Asp Glu Tyr Glu Asp Glu Thr Phe Glu  
210 215 220

Lys Gln Leu Glu Asp Ile Phe Ala Gln Ile Arg Pro Leu Tyr Glu Gln  
225 230 235 240

Leu His Ala Tyr Val Arg Phe Lys Leu Arg Glu Lys Tyr Gly Asn Asp  
245 250 255

Val Val Ser Glu Lys Gly Pro Ile Pro Met His Leu Leu Gly Asn Met  
260 265 270

Trp Gly Gln Thr Trp Ser Glu Val Ala Pro Ile Leu Val Pro Tyr Pro  
275 280 285

Glu Lys Lys Leu Leu Asp Val Thr Asp Glu Met Val Lys Gln Gly Tyr  
290 295 300

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

Asn Met Thr Lys Leu Pro Lys Thr Phe Trp Glu Tyr Ser Ile Leu Glu  
325 330 335

Lys Pro Gln Asp Gly Arg Glu Leu Ile Cys His Ala Ser Ala Trp Asp  
340 345 350

Phe Tyr Thr Lys Asp Asp Val Arg Lys Gln Cys Thr Arg Val Thr Met  
355 360 365

Asp Gln Phe Phe Thr Ala His His Glu Leu Gly His Ile Gln Tyr Tyr  
370 375 380

Leu Gln Tyr Gln His Leu Pro Ser Val Tyr Arg Glu Gly Ala Asn Pro  
385 390 395 400

Gly Phe His Glu Ala Val Gly Asp Val Leu Ser Leu Ser Val Ser Ser  
405 410 415

Pro Lys His Leu Glu Lys Val Gly Leu Leu Lys Asp Phe Lys Phe Asp  
420 425 430

Glu Glu Ser Gln Ile Asn Gln Leu Leu Asn Leu Ala Leu Asp Lys Met  
435 440 445

Ala Phe Leu Pro Phe Ala Tyr Thr Ile Asp Lys Tyr Arg Trp Gly Val  
450 455 460

Phe Arg Gly Glu Ile Ser Pro Ser Glu Tyr Asn Cys Lys Phe Trp Glu  
465 470 475 480

Met Arg Ser Tyr Tyr Gly Ile Glu Pro Pro Ile Ala Arg Ser Glu  
485 490 495

Ser Asp Phe Asp Pro Pro Ala Lys Tyr His Ile Ser Ser Asp Val Glu  
500 505 510

Tyr Leu Arg Tyr Leu Val Ser Phe Ile Ile Gln Phe Gln Phe His Gln  
515 520 525

Ala Val Cys Gln Lys Thr Gly Gln Phe Val Pro Asn Asp Pro Glu Lys  
530 535 540

Thr Leu Leu Asn Cys Asp Ile Tyr Gln Ser Ala Glu Ala Gly Asn Ala  
545 550 555 560

Phe Lys Glu Met Leu Lys Leu Gly Ser Ser Lys Pro Trp Pro Asp Ala  
565 570 575

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

Glu Tyr Phe Arg Pro Leu Ser Glu Trp Leu Gln Lys Lys Asn Lys Glu  
 595 600 605

Leu Gly Ala Tyr Val Gly Trp Asp Lys Ser Thr Lys Cys Val Lys Asn  
 610 615 620

Val Ser  
 625

<210> 44  
 <211> 2121  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 44  
 gtatatcaag tatcattcaa gtgaatcatt ggctccgtaa tttgtacaaa agaaaaaaaaa 60  
  
 gagtagtgct gtgcctaaga aaaacggtga aactattgaa gaagaagaag taagagctga 180  
 gcaacgactt agagagatca atgaggaact tgatcgtagg aagaatatca atactgtac 240  
 cgcttggct tatgcattcca atattactga ggtcaatctc aagaacatga atgatgtgtc 300  
 ggttgaaacc gcgaaatact acaaggaact tgcattgtgaa ttgaaggat tcaatgccaa 360  
 ggaataacaag agtgaggatc tgaagagaca aattaagaag ctaagcaagt tggatata 420  
 tgcttacca tctgagaagt ataaggagct ttggagact atcacatgga tggatcgaa 480  
 ttatgcaaaa gtgaaagttt gctcatacaa ggatccaaag aaatgtgatt tagcacttga 540  
 acctgaaatt acggaaatcc ttattaaag tcgagatcct gaggaactta aatattattg 600  
 gaaacaatgg tacgacaaag ctggcacacc aactcgagag agtttaata agtatgtaca 660  
 actaaatcgtaa gaagcagcga aattggatgg attttattcg ggtgcagaat cttggcttga 720  
 tgaatatgaa gatgagacat ttgagaaaca acttgaggat atcttcgccc aaattcgccc 780  
 actgtacgag caactccatg cttatgttag attcaagctg agggaaaagt atggaaatga 840  
 cgttgttgcg gagaaggtc ccattccat gcatctcttggaaacatgt ggggtcaaac 900  
 gtggagtgaa gttgccccaa ttttagtccc ataccccgaa aagaagctcc tcgatgttac 960  
 cgatgagatg gttaagcagg gatacacacc aatttctatg tttgaaaaag gagacgaatt 1020  
 tttccaaagc ttgaatatga cgaaacttcc aaaaaccccttc tgggagtgaca gtatggat 1080  
 aaaaccccaa gatggtaggg aattgatctg ccatgcaagt gcatggact totatacaaa 1140  
 ggtatgtta aggattaaac agtgtaccag agttacaatg gatcaattct tcacggctca 1200  
 tcatgagctt ggtcacattc aatattatgtt gcaatatcaa catttgcgaa gtgtttacag 1260  
 agaagggtgcc aatccaggct ttcacgaggc tggggat gttctcttc tttcggtatc 1320

aagtccctaaa catttggaaa aagttggttt gcttaaagac ttcaaatttg atgaagaatc	1380
ccagataaaat caacttctaa atttagctct ggataaaaatg gcattcctcc catttgccta	1440
taccatttgcgtt aaatatcgct ggggtgtgtt tcgggggtgaa atttcgccgt ctgagtacaa	1500
ttgcaaattt tggaaaatgc gttcctacta tgggtgtata gaaccaccaa ttgcacgttc	1560
tgagagtgtat ttgatccac cagcaaaata tcatatttca tcggatgtt agtacctcag	1620
gtatttggtt tccttcatta ttcagttcca attccatcaa gctgtgtgcc aaaagactgg	1680
tcagttcgta ccgaatgatc cggagaagac tcttctaaat tgtgacatct accagagtgc	1740
tgaggctggt aatgccttca aagaaatgct caaattggga tcctcaaaac catggccaga	1800
tgcaatggaa attcttacgg ggcaaaaggaa aatggatgct tctgcattaa ttgagtactt	1860
ccgtccactc agtgagtggc tgcagaagaa gaataaggaa ctaggagctt atgttggctg	1920
ggacaaatct actaagtgtg tcaaaaacgt cagtttattt tttgtgagcc ctaaaaaata	1980
ttcataacat ttcaatatga caaaatataat gatttcgtg aaaactaagc atgagtaagt	2040
ttttttgtg aatttttagc agtttcattt cagaataaac gtcaaatttt taaaaaaaaaa	2100
aaaaaaaaaaa aaaaaaaaaaa a	2121

&lt;210&gt; 45

&lt;211&gt; 42

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 45

Met Lys Thr Phe Ala Leu Ile Phe Leu Ala Leu Ala Val Phe Val Leu			
1	5	10	15

Cys Ile Asp Gly Ala Pro Thr Phe Val Asn Leu Leu Asp Asp Val Gln		
20	25	30

Glu Glu Val Glu Val Asn Thr Tyr Glu Pro	
35	40

&lt;210&gt; 46

&lt;211&gt; 463

&lt;212&gt; DNA

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 46

tcagtttagtt gactaacaaa ccacaataga gacactaaaa tgaagacatt cgcccttaatc	60
ttcttggctc ttgctgtttt tgtgctctgc attgacggag ctccaaacttt tgtgaattta	120
ctggacgacg tacaggaaga ggtagaagtt aatacgtatg agccttagga agaaaatgtt	180
tgaggagttt caggcagagg cagagcttcc ccagagaggg agctttgcc ttgctgtaga	240

tttttaaaaa tgaatcaatt tgattggagc aattacgcta tatttgtgg aatatttttg 300  
 aattaaaaac taattatgga aattaatata taatttcag aatttcaata aattcatcaa 360  
 aattgtatta attaaaaat attgtatgaa attcccaata aaagcttca aattaaaaaa 420  
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 463

<210> 47  
 <211> 139  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 47

Met Asn His Leu Cys Phe Ile Ile Ile Ala Leu Phe Phe Leu Val Gln  
 1 5 10 15

Gln Ser Leu Ala Glu His Pro Glu Glu Lys Cys Ile Arg Glu Leu Ala  
 20 25 30

Arg Thr Asp Glu Asn Cys Ile Leu His Cys Thr Tyr Ser Tyr Tyr Gly  
 35 40 45

Phe Val Asp Lys Asn Phe Arg Ile Ala Lys Lys His Val Gln Lys Phe  
 50 55 60

Lys Lys Ile Leu Val Thr Phe Gly Ala Val Pro Lys Lys Glu Lys Lys  
 65 70 75 80

Lys Leu Leu Glu His Ile Glu Ala Cys Ala Asp Ser Ala Asn Ala Asp  
 85 90 95

Gln Pro Gln Thr Lys Asp Glu Lys Cys Thr Lys Ile Asn Lys Tyr Tyr  
 100 105 110

Arg Cys Val Val Asp Gly Lys Ile Leu Pro Trp Asn Ser Tyr Ala Asp  
 115 120 125

Ala Ile Ile Lys Phe Asp Lys Thr Leu Asn Val  
 130 135

<210> 48  
 <211> 579  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 48  
 ggccattatg gccggggata gaacttaatt gttgttaaaa tgaatcactt gtgctttatt 60

attattgctc tattctttt ggttcaacaa tccttggctg aacatccaga agaaaaatgt	120
attagagaat tggcgagaac tgatgaaaac tgcattctc attgtacgta ttcgtactac	180
ggattcgttg ataaaaaattt caggatcgct aaaaaacatg ttcaaaaatt caaaaaaaatc	240
ctagttacat tcggcgctgt tcctaagaaa gaaaaaaaga aactttaga gcacattgag	300
gcttgtgcgg attctgcgaa tgctgatcaa cctcaaaacta aagatgaaaa atgtacaaaa	360
ataaataagt actatcgttg tggatggat ggaaaaatata taccctggaa tagttatgct	420
gatgcaatca ttaagttga taaaaccctt aacgtatgaa gcaaagatata tcgaaaaaaaa	480
aacatcaaga ttatgctgga aagaaaaaaaaaaa ttgtgctaataa caaattgaat	540
taacgcttaa tgctatatta aaaaaaaaaaaa aaaaaaaaaaaa	579

&lt;210&gt; 49

&lt;211&gt; 446

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 49

Met Lys Ile Ile Phe Leu Ala Ala Phe Leu Leu Ala Asp Gly Ile Trp	
1	5
	10
	15

Ala Ala Glu Glu Pro Ser Val Glu Ile Val Thr Pro Gln Ser Val Arg	
20	25
	30

Arg His Ala Thr Pro Lys Ala Gln Asp Ala Arg Val Gly Ser Glu Ser	
35	40
	45

Ala Thr Thr Ala Pro Arg Pro Ser Glu Ser Met Asp Tyr Trp Glu Asn	
50	55
	60

Asp Asp Phe Val Pro Phe Glu Gly Pro Phe Lys Asp Ile Gly Glu Phe	
65	70
	75
	80

Asp Trp Asn Leu Ser Lys Ile Val Phe Glu Glu Asn Lys Gly Asn Ala	
85	90
	95

Ile Leu Ser Pro Leu Ser Val Lys Leu Leu Met Ser Leu Leu Phe Glu	
100	105
	110

Ala Ser Ala Ser Gly Thr Leu Thr Gln His Gln Leu Arg Gln Ala Thr	
115	120
	125

Pro Thr Ile Val Thr His Tyr Gln Ser Arg Glu Phe Tyr Lys Asn Ile	
130	135
	140

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

Thr Arg Ile Tyr Val Asp Gln Phe Val Thr Pro Arg Gln Arg Tyr Ala  
165 170 175

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

Ser Lys Ala Lys Glu Thr Thr Gln Ala Ile Asn Ser Trp Val Ser Asn  
195 200 205

Ile Thr Asn Glu His Ile Lys Asp Leu Val Lys Glu Glu Asp Val Gln  
210 215 220

Asn Ser Val Met Leu Met Leu Asn Ala Val Tyr Phe Arg Gly Leu Trp  
225 230 235 240

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

Asp Glu Ser Lys Thr Thr Asp Phe Met Leu Thr Asp Gly Leu Tyr Tyr  
260 265 270

Phe Tyr Glu Ala Lys Glu Leu Asp Ala Lys Ile Leu Arg Ile Pro Tyr  
275 280 285

Lys Gly Lys Gln Tyr Ala Met Thr Val Ile Leu Pro Asn Ser Lys Ser  
290 295 300

Gly Ile Asp Ser Phe Val Arg Gln Ile Asn Thr Val Leu Leu His Arg  
305 310 315 320

Ile Lys Trp Leu Met Asp Glu Val Glu Cys Arg Val Ile Leu Pro Lys  
325 330 335

Phe His Phe Asp Met Thr Asn Glu Leu Lys Glu Ser Leu Val Lys Leu  
340 345 350

Gly Ile Ser Gln Ile Phe Thr Ser Glu Ala Ser Leu Pro Ser Leu Ala  
355 360 365

Arg Gly Gln Gly Val Gln Asn Arg Leu Gln Val Ser Asn Val Ile Gln  
370 375 380

Lys Ala Gly Ile Ile Val Asp Glu Lys Gly Ser Thr Ala Tyr Ala Ala  
 385 390 395 400

Ser Glu Val Ser Leu Val Asn Lys Phe Gly Asp Asp Glu Phe Val Met  
 405 410 415

Phe Asn Ala Asn His Pro Phe Leu Phe Thr Ile Glu Asp Glu Thr Thr  
 420 425 430

Gly Ala Ile Leu Phe Thr Gly Lys Val Val Asp Pro Thr Gln  
 435 440 445

<210> 50  
 <211> 1651  
 <212> DNA  
 <213> Lutzomyia longipalpis

<220>  
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 ttaagtgtgt gtgtcttagc aaagtgattt ccacaatgaa gattattttt ttagccgctt 120  
 ttctactagc ggatggattt tgggctgctg aagaaccttc agtggaaatt gtaacaccac 180  
 aatcagtgcg gagacacgct acgccaaaag cccaggacgc gagggtagga agtgaatccg 240  
 caacaacagc accaagacca agtgaatcaa tggattactg ggagaatgat gattcgtcc 300  
 catttgaggg tccattcaag gatattggag aattcgactg gaacctttcg aagatcgaaa 360  
 ttgagggaaa caaaaggtaat gccatcttgt cgcactctc tgtgaagcta ctaatgagtt 420  
 tgctcttcga ggccagtgcg tcaggtacct tgacccagca ccaactcaga caagccactc 480  
 ccaccatcgt caccactat cagtctcgag aattttacaa gaatatctt gacggctctca 540  
 agaaaaagag taacgactac acggttcact ttggtagcag aatctacgtg gatcagttt 600  
 tgacgcctcg ccagagatat gctgccattt tggagaagca ttatctgact gatctcaaag 660  
 ttgaggactt ctgcaggca aaagaaacaa ctcaggcaat caatagttgg gtgtcaaaca 720  
 tcacaaatga gcacataaag gatctcgta aggaggaaga tggatcagaat tcaagttatgc 780  
 tcatgcttaa tgcagtctac ttccgcggac tctggcgcaa gccttcaat cgtacactcc 840  
 cactgccctt ccacgtgagc gctgatgagt ccaagacgac tgattttatg ctaaccgatg 900  
 ggctctacta cttctacgag gcaaaggaaat tggatgctaa gatcctcaga attccttaca 960  
 aaggtaaaca atacgcaatg actgtgatct taccaaattc caagagtggc attgatagct 1020

ttgtgcgtca gattaacacg gtcctcctgc acaggattaa gtggttgatg gatgaagtgg 1080  
 agtgcagggt tattctaccc aagttccact ttgacatgac gaatgagctg aaggaatcgc 1140  
 tcgtaaagtt gggcatcagt cagatttca catcagaggc atcttgcca tcattagcac 1200  
 gaggacaggg cgtacagaat cgtctgcagg tgtctaatgt gattcagaag gcgggataaa 1260  
 ttgtggatga gaagggcago acagccatcg ctgcgtcaga agtgaggccta gtcaacaagt 1320  
 ttggagatga ttagttcgta atgttcaacg ctaatcatcc attcctctt acaattgagg 1380  
 acgaaaccac cggcgcaatc ctatcacgg gaaaagtcgt cgatcccacg caatagggaa 1440  
 tgaaaagcat ttcatcgtat acaacttttt tttaattaa ttattcctca ttgaaggaca 1500  
 ttaatagagc atcttctcag gaaggcactc ctgacttatt tttactaaat gtgatccttg 1560  
 gacacataaa aaaaacagct gtacttctta cttttataaa tatacgacca tatttgtgag 1620  
 gaaaaaaaaa aaaaanaaaaa aaaaaaaaaa a 1651

<210> 51

<211> 166

<212> PRT

<213> Lutzomyia longipalpis

<400> 51

Met Arg Phe Leu Leu Leu Ala Phe Ser Val Ala Leu Val Leu Ser Pro  
 1 5 10 15

Thr Phe Ala Lys Pro Gly Leu Trp Asp Ile Val Thr Gly Ile Asn Asp  
 20 25 30

Met Val Lys Asn Thr Ala Asn Ala Leu Lys Asn Arg Leu Thr Thr Ser  
 35 40 45

Val Thr Leu Phe Thr Asn Thr Ile Thr Glu Ala Ile Lys Asn Ala Asn  
 50 55 60

Ser Ser Val Ser Glu Leu Leu Gln Gln Val Asn Glu Thr Leu Thr Asp  
 65 70 75 80

Ile Ile Asn Gly Val Gly Gln Val Gln Ser Ala Phe Val Asn Ser Ala  
 85 90 95

Gly Asn Val Val Val Gln Ile Val Asp Ala Ala Gly Asn Val Leu Glu  
 100 105 110

Val Val Val Asp Glu Ala Gly Asn Ile Val Glu Val Ala Gly Thr Ala  
 115 120 125

Leu Glu Thr Ile Ile Pro Leu Pro Gly Val Val Ile Gln Lys Ile Ile  
 130 135 140

Asp Ala Leu Gln Gly Asn Ala Gly Thr Thr Ser Asp Ser Ala Ser Ser  
 145 150 155 160

Thr Val Pro Gln Gln Ser  
 165

<210> 52  
 <211> 739  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 52  
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 aactggtatt aatgatatgg taaaaaatac tgcgaatgca ctcaaaaatc gtctaacaac 180  
 ttctgtgaca ttattcacaa ataccatcac cgaagctata aaaaatgcaa attcttctgt 240  
 ttcggaactc cttcagcaag tcaatgaaac ccttacggat attattaatg gtgttaggaca 300  
 agtgcagagt gcctttgtga attcagctgg aaatgttgtt gtgcaaattg ttgatgccgc 360  
 tggaaatgtt ttggaagttt ttgttgatga ggctggaaat atcgtggagg tagctggAAC 420  
 agcattggaa actatcatc cactgcccgg ttagtgatt cagaagataa ttgatgctct 480  
 ccaaggaaat gcagggacta catcgatttc agcttcatca actgtcccc aacaatctta 540  
 actacaaccg caatgatgtt gtcttaacg gagaattttt aaatttgaat atcaaaatcc 600  
 aagatgaaat attcagattt ttcaatcaat atgatacgaa attttgaaat tattttccg 660  
 actaaagcaa tttgtaaaag gaaaacccaa taaatatttgg aaattgtaaa gaaaaaaaaa 720  
 aaaaaaaaaa aaaaaaaaaa 739

<210> 53  
 <211> 109  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 53

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

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

Ala Lys Tyr Leu Gln Asp Glu Cys Asn Ala Gly Glu Ile Ala Asp Glu  
 35 40 45

Phe Leu Pro Phe Ser Glu Glu Glu Val Gly Glu Ala Leu Ser Asp Lys  
 50 55 60

Pro Glu Asn Val Gln Glu Val Thr Asn Ile Val Arg Gly Cys Phe Glu  
 65 70 75 80

Ala Glu Gln Ala Lys Glu His Gly Lys Cys Glu Arg Phe Ser Ala Leu  
 85 90 95

Ser Gln Cys Tyr Ile Glu Lys Asn Leu Cys Gln Phe Phe  
 100 105

<210> 54

<211> 447

<212> DNA

<213> Lutzomyia longipalpis

<400> 54

atatcaattt tatacatcatg gtgaagtact cgtgtttgt tcttggca attttttttc 60

tggccggacc ctacggcggtt gtaggttctt gtgagaatga cctgacagag gcccggcaagt 120

atcttcaaga tgaatgcaat gcaggtgaaa ttgcagatga atttctaccc ttctctgaag 180

aagaagtggg tgaagcattt agcgacaaac cagaaaacgt gcaggaagtc accaacatcg 240

tgagaggatg ctttgaagct gaacaagcca aagagcatgg aaaatgtgaa agatttccg 300

ctttgagtca atgctacatt gaaaagaatt tatgtcaatt cttctaaaat attttgaaga 360

aaagttatga atgaaaattt tctgaaattt tggcaaaa atatataaaat tgcccaattt 420

aaaaaaaaaaaa aaaaaaaaaaa aaaaaaaaaa 447

<210> 55

<211> 115

<212> PRT

<213> Lutzomyia longipalpis

<400> 55

Met Lys Phe Phe Tyr Leu Ile Phe Ser Ala Ile Phe Phe Leu Ala Asp  
 1 5 10 15

Pro Ala Leu Val Lys Cys Ser Glu Asp Cys Glu Asn Ile Phe His Asp  
 20 25 30

Asn Ala Tyr Leu Leu Lys Leu Asp Cys Glu Ala Gly Arg Val Asp Pro  
 35 40 45

Val Glu Tyr Asp Asp Ile Ser Asp Glu Glu Ile Tyr Glu Ile Thr Val  
 50 55 60

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

Arg Glu Cys Ile Ala Gln Val Ser Thr Gln Asp Cys Thr Lys Phe Ser  
 85 90 95

Glu Ile Tyr Asp Cys Tyr Met Lys Lys Ile Cys Asn Tyr Tyr Pro  
 100 105 110

Glu Asn Met  
 115

<210> 56  
 <211> 496  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 56  
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 gatcctgctt tggcaagtg ttcagaggat tgtgagaata ttttcatga caatgcgtac 120  
 ctccttaaat tggattgtga agcaggaagg gttgatcctg ttgaatacga cgatatttcg 180  
 gatgaagaaa tatatgaaat aacggtcgtat gttggagttt catctgagga ccaggagaaa 240  
 gttgcgaaaaa taataaggaa gtgcattgca caagtttcaa cgcaagattt cacgaaattt 300  
 tcagaaattt atgattgtta catgaagaag aaaatctgtt attattatcc tgaaaatatg 360  
 taaaaaaaaa ttatttattt atataaaaaa atataaggat taaaatctct tattgattgt 420  
 aaaaatggcc taatattgaa gcaaaaatta aagcatgaaa caagacccaaa aaaaaaaaaa 480  
 aaaaaaaaaa aaaaaa 496

<210> 57  
 <211> 409  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 57

Met His Leu Gln Leu Asn Leu Cys Ala Ile Leu Leu Ser Val Leu Asn  
 1 5 10 15

Gly Ile Gln Gly Ala Pro Lys Ser Ile Asn Ser Lys Ser Cys Ala Ile  
 20 25 30

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

Pro Ser Asn Asp Gly Ser Leu Ser Thr Pro Leu Gln Pro Ser Gly Pro  
50 55 60

Phe Val Ser Leu Lys Ile Gly Glu Ser Leu Ala Ile Phe Cys Pro Gly  
65 70 75 80

Asp Gly Lys Asp Val Glu Thr Ile Thr Cys Asn Thr Asn Phe Asp Leu  
85 90 95

Ala Ser Tyr Ser Cys Asn Lys Ser Thr Ser Thr Asp Thr Ile Glu Thr  
100 105 110

Glu Glu Val Cys Gly Gly Ser Gly Lys Val Tyr Lys Val Gly Phe Pro  
115 120 125

Leu Pro Ser Gly Asn Phe His Ser Ile Tyr Gln Thr Cys Phe Asp Lys  
130 135 140

Lys Asn Leu Thr Pro Leu Tyr Ser Ile His Ile Leu Asn Gly Gln Ala  
145 150 155 160

Val Gly Tyr His Leu Lys His Thr Arg Gly Ser Phe Arg Thr Asn Gly  
165 170 175

180 185 190

Lys Phe Asn Lys Leu Phe Gly Pro Lys Gln Thr Phe Phe Arg Arg Pro  
195 200 205

Leu Asn Phe Leu Ser Arg Gly His Leu Ser Pro Glu Val Asp Phe Thr  
210 215 220

Phe Arg Arg Glu Gln His Ala Thr Glu Met Tyr Ile Asn Thr Ala Pro  
225 230 235 240

Gln Tyr Gln Ser Ile Asn Gln Gly Asn Trp Leu Arg Val Glu Asn His  
245 250 255

Val Arg Asp Leu Ala Lys Val Leu Gln Lys Asp Ile Thr Val Val Thr  
260 265 270

Gly Ile Leu Gly Ile Leu Arg Leu Lys Ser Lys Lys Ile Glu Lys Glu

275

280

285

Ile Tyr Leu Gly Asp Asp Val Ile Ala Val Pro Ala Met Phe Trp Lys  
 290 295 300

Ala Val Phe Asp Pro Gln Lys Gln Glu Ala Ile Val Phe Val Ser Ser  
 305 310 315 320

Asn Asn Pro His Val Lys Thr Phe Asn Pro Asn Cys Lys Asp Val Cys  
 325 330 335

Ala Gln Ala Gly Phe Gly Asn Asp Asn Leu Glu Tyr Phe Ser Asn Tyr  
 340 345 350

Ser Ile Gly Leu Thr Ile Cys Cys Lys Leu Glu Glu Phe Val Lys Arg  
 355 360 365

Asn Lys Ile Ile Leu Pro Lys Glu Val Asn Asn Lys Asn Tyr Thr Lys  
 370 375 380

Lys Leu Leu Lys Phe Pro Lys Thr Arg Asn Lys Glu Gly Asp Lys Lys  
 385 390 395 400

Val Val Arg Lys Arg Ala Lys Gly Ala  
 405

<210> 58

<211> 1281

<212> DNA

<213> Lutzomyia longipalpis

<400> 58

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atgtaacggc	taagaaggag	ccagtgtact	tgaaaccatc	aatgatggc	tcattgagta	180
ccccctaca	gccaaagtggg	ccatttgtaa	gtctcaaaat	tggagaatct	cttgcattct	240
tctgtccagg	tgatggaaag	gacgtagaga	caattacgtg	caatacaa	ttcgatttag	300
cttcatattc	gtgcaacaag	agcacatcaa	cggataccat	tgaaacggaa	gaagtttgcg	360
gaggaagtgg	aaaagtgtac	aaagttggtt	ttccgctgcc	ctctggaaat	ttccattcaa	420
tctaccaa	gtgtttgtat	aagaaaaatc	tcacacctct	ctactcaatt	cacattctca	480
atggtaagc	tgttggat	cacctaagc	acacaagagg	aagcttcgt	accaatggta	540
tctacgggaa	agtcaacatt	gataaactct	acaagacgca	aattgagaaa	ttcaacaaac	600

ttttcggccc taaacaaaaca tttttccgta gaccctcaa ttttctatca cgtggacact 660  
 taagccccga agtggacttt acattccgta gggacaaca tgcaacggaa atgtacatta 720  
 acacagcacc acagtaccaa tcaattaatc aaggaaattg gctacgttt gaaaatcacg 780  
 tgagggatct cgcaaaaagtt ctgcagaagg acataacagt cgttacggga attttgggga 840  
 tacttcggtt gaagagtaag aaaatagaga aagaaatcta ttttaggagat gacgtaattg 900  
 ccgtaccagc aatgttctgg aaggctgtt ttgaccctca aaaacaagaa gcaattgtct 960  
 ttgtttcctc aaataatccc cacgtgaaga cctttaatcc caactgcaag gatgtatgctg 1020  
 ctcaagctgg atttggaaat gataatcttgc aatatttctc caattattctt attggctctga 1080  
 ctattttttg caaacttgag gaatttgtta aaagaaataa aataattcta cccaaagaag 1140  
 taaataacaa aaactacacc aaaaaactcc ttaagttcc taaaacaaga aacaaggagg 1200  
 gagataagaa ggtggtaacgt aagcgcgc aaggagcata aatattaaac gaaaaaaaaa 1260  
 aaaaaaaaaa aaaaaaaaaa a 1281

&lt;210&gt; 59

&lt;211&gt; 160

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 59

Met Asn Leu His Leu Ala Ile Ile Leu Phe Val Ser Tyr Phe Thr Leu  
 1 5 10 15

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

Ile Phe Ile Ser Lys Ala Glu Leu Thr Trp Phe Gln Ala Leu Asp Phe  
 35 40 45

Cys Thr Glu Gln Asn Leu Thr Leu Leu Ser Ile Lys Ser Ala Arg Glu  
 50 55 60

Asn Asp Glu Val Thr Lys Ala Val Arg Ala Glu Val His Leu Pro Asp  
 65 70 75 80

Thr Lys Lys Ser His Ile Trp Leu Gly Gly Ile Arg Tyr Asp Gln Asp  
 85 90 95

Lys Asp Phe Arg Trp Ile Ser Asp Gly Thr Thr Val Thr Lys Thr Val  
 100 105 110

Tyr Ile Asn Trp Tyr Gln Gly Glu Pro Asn Gly Gly Arg Tyr Gln Lys

115	120	125
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Glu	Phe	Cys	Met	Glu	Leu	Tyr	Phe	Lys	Thr	Pro	Ala	Gly	Gln	Trp	Asn
130	135	140													

Asp	Asp	Ile	Cys	Thr	Ala	Lys	His	His	Phe	Ile	Cys	Gln	Glu	Lys	Lys
145					150					155				160	

<210> 60  
 <211> 671  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 60	60														
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aaatgaattt	gcaccttgcg	attatcctct	ttgtgagtt	cttcacactg	atcactgcta										
cggatctaat	tgaaaaggaa	ctttctgatt	gcaaaaagat	cttcatctcc	aaggctgagc										
taacttggtt	ccaaagctctc	gatttctgt	ccgaacaaaa	cctaactttg	ctctcaatta	taatccgccc	ggaaaatgt	gaggtgacta	aagcagttcg	agctgaggtt	catcttccag	catcttccag	catcttccag	catcttccag	catcttccag
aatccgccc	gtctcacatt	tggctcgag	gtattcgta	tgatcaagac	aaggatttcc										
gttggataag	cgatggaaca	actgttacga	agacagtcta	catcaattgg	taccaaggag										
aaccaaagaa	gtctcacatt	tggctcgag	gtattcgta	tgatcaagac	aaggatttcc										
ctggtaatg	gaatgatgat	atttgtacag	caaagcatca	ttttatatgt	caggagaaaa	aataaaatgg	attgttcatg	tgtcttggc	ggtgcgaagg	tataattcg	gttgacgaca	gttgacgaca	gttgacgaca	gttgacgaca	gttgacgaca
aataaaatgg	tttctttcat	taagaaaata	aaggcttgaa	tttataaaaa	aaaaaaaaaa										
aaaaaaaaaa	a														671

<210> 61  
 <211> 160  
 <212> PRT  
 <213> Lutzomyia longipalpis

<400> 61

Met	Asn	Leu	Pro	Leu	Ala	Ile	Ile	Leu	Phe	Val	Ser	Tyr	Phe	Thr	Leu
1				5				10				15			

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

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

Cys Thr Glu Lys Asp Leu Thr Leu Leu Thr Ile Lys Ser Ala Arg Glu  
 50 55 60

Asn Glu Glu Val Thr Lys Ala Val Arg Ala Glu Val His Leu Pro Asp  
 65 70 75 80

Thr Lys Lys Ser His Ile Trp Leu Gly Gly Ile Arg Tyr Asp Gln Asp  
 85 90 95

Lys Asp Phe Arg Trp Ile Ser Asp Gly Thr Thr Val Thr Lys Thr Val  
 100 105 110

Tyr Ile Asn Trp Tyr Gln Gly Glu Pro Asn Gly Gly Arg Tyr Gln Lys  
 115 120 125

Glu Phe Cys Met Glu Leu Tyr Phe Lys Thr Pro Ala Gly Gln Trp Asn  
 130 135 140

Asp Asp Ile Cys Thr Ala Lys His His Phe Ile Cys Gln Glu Lys Lys  
 145 150 155 160

<210> 62  
 <211> 672  
 <212> DNA  
 <213> *Lutzomyia longipalpis*

<400> 62  
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 aaatgaattt gccccttgcg attatcctct ttgtgagttt cttcacactg atcactgctg 120  
 cggatctaac tgaaaaggaa ctttctgatg gcaaaaagat cttcatctcc aaggctgagc 180  
 taagttggtt cgatgctctc gatgcctgta ccgaaaaaga cctaactttt ctcacaatta 240  
 aatccgccccg ggaaaatgag gaagtgacta aagcagttcg agctgaggtt catcttccag 300  
 acacaaagaa gtctcacatt tggctggag gtattcgta tcatcaagac aaggatttcc 360  
 gttggataag cgatggaaca actgttacga agacagtcta catcaattgg taccaggag 420  
 aaccaaatgg tgggaggtac caaaaaggaat tttgtatgga attgtacttt aaaactccag 480  
 ctggtaatg gaatgatgat atttgcacatg caaagcatca ttttatatgt caggagaaaa 540  
 aataaaattga attgttcatg tgtcttggc ggtgcgaagg tataattcag gttgacgaca 600  
 taaaattgatt tttcttcat taagaaaata aaggcttcaa ttttagcaaaa aaaaaaaaaaa 660  
 aaaaaaaaaaa aa 672

<210> 63  
 <211> 399

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 63

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

Leu Gly Ala Asp Thr Gln Gly Tyr Lys Trp Lys Gln Leu Leu Tyr Asn  
20 25 30

Asn Val Thr Pro Gly Ser Tyr Asn Pro Asp Asn Met Ile Ser Thr Ala  
35 40 45

Phe Ala Tyr Asp Ala Glu Gly Glu Lys Leu Phe Leu Ala Val Pro Arg  
50 55 60

Lys Leu Pro Arg Val Pro Tyr Thr Leu Ala Glu Val Asp Thr Lys Asn  
65 70 75 80

Ser Leu Gly Val Lys Gly Lys His Ser Pro Leu Leu Asn Lys Phe Ser  
85 90 95

Gly His Lys Thr Gly Lys Glu Leu Thr Ser Ile Tyr Gln Pro Val Ile  
100 105 110

Asp Asp Cys Arg Arg Leu Trp Val Val Asp Ile Gly Ser Val Glu Tyr  
115 120 125

Arg Ser Arg Gly Ala Lys Asp Tyr Pro Ser His Arg Pro Ala Ile Val  
130 135 140

Ala Tyr Asp Leu Lys Gln Pro Asn Tyr Pro Glu Val Val Arg Tyr Tyr  
145 150 155 160

Phe Pro Thr Arg Leu Val Glu Lys Pro Thr Tyr Phe Gly Gly Phe Ala  
165 170 175

Val Asp Val Ala Asn Pro Lys Gly Asp Cys Ser Glu Thr Phe Val Tyr  
180 185 190

Ile Thr Asn Phe Leu Arg Gly Ala Leu Phe Ile Tyr Asp His Lys Lys  
195 200 205

Gln Asp Ser Trp Asn Val Thr His Pro Thr Phe Lys Ala Glu Arg Pro  
210 215 220

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

Phe Gly Ile Thr Leu Gly Asp Arg Asp Ser Glu Gly Asn Arg Pro Ala  
 245 250 255

Tyr Tyr Leu Ala Gly Ser Ala Ile Lys Val Tyr Ser Val Asn Thr Lys  
 260 265 270

Glu Leu Lys Gln Lys Gly Gly Lys Leu Asn Pro Glu Leu Leu Gly Asn  
 275 280 285

Arg Gly Lys Tyr Asn Asp Ala Ile Ala Leu Ala Tyr Asp Pro Lys Thr  
 290 295 300

Lys Val Ile Phe Phe Ala Glu Ala Asn Thr Lys Gln Val Ser Cys Trp  
 305 310 315 320

Asn Thr Gln Lys Met Pro Leu Arg Met Lys Asn Thr Asp Val Val Tyr  
 325 330 335

Thr Ser Ser Arg Phe Val Phe Gly Thr Asp Ile Ser Val Asp Ser Lys  
 340 345 350

Gly Gly Leu Trp Phe Met Ser Asn Gly Phe Pro Pro Ile Arg Lys Ser  
 355 360 365

Glu Lys Phe Lys Tyr Asp Phe Pro Arg Tyr Arg Leu Met Arg Ile Met  
 370 375 380

Asp Thr Gln Glu Ala Ile Ala Gly Thr Ala Cys Asp Met Asn Ala  
 385 390 395

<210> 64  
 <211> 1429  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 64  
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 agggaccctt ggagcggata ctcaaggata taaatggaag caattgctct acaataatgt 120  
 tacaccagga tcctacaatc cggataatat gatcagtacg gctttgcct acgatgctga 180  
 gggtgaaaaa ctcttcctag ctgtcccaag gaagttaccc agagttccgt atacattggc 240  
 ggaagtggat acaaagaata gtcttggtgt taaggaaaa cattcaccgt tacttaacaa 300  
 attcagtggg cacaactg ggaaggaact aacatcaatc tatcagccag ttattgatga 360

ttgtcgctcg	cttgggtgg	ttgatattgg	ttccgtggaa	tatcgctcaa	gaggtgccaa	420
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cgaagttgtt	cgatactatt	tccccacaag	attagtggag	aagccaacat	atttcgggtgg	540
atttgcgtt	gatgttgcaa	acccaaaggg	ggattgttagt	gaaactttg	tctacattac	600
aaacttcctc	aggggagctc	tctttatata	cgatcataag	aagcaggatt	cgtggaatgt	660
aactcatccc	acttcaaag	cagaacgacc	cactaaattt	gattacggcg	gaaaggaata	720
tgaattcaa	gccgaaattt	tcggaattac	tctcgagat	cgagacagt	aaggcaatcg	780
tccagcttac	tacttagccg	gaagtgccat	caaagtctac	agcgtcaaca	cgaagaact	840
taagcagaag	ggtggaaagc	tgaatccgga	gcttcttgg	aaccgcggga	agtacaacga	900
tgccattgcc	ctagcttacg	atccaaaac	taaagttatc	ttctttgctg	aggccaacac	960
aaagcaagta	tcctgctgga	acacacagaa	aatgccactg	aggatgaaga	ataccgacgt	1020
agtctacact	agttctcg	ttgtcttgg	aacggacatt	tcgggtgata	gcaaggcgg	1080
cctctggttc	atgtctaacg	gcttccgccc	tataaggaaa	tcagaaaaat	tcaaatatga	1140
cttcccacgc	taccgtctaa	tgaggatcat	ggacacacag	gaagcaattt	ccggactgc	1200
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aggagaaattt	attgattctg	aattctataa	aaaaaaattt	atttgtgaaa	tatggcaaa	1380
taataaaat	attgaattac	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	1429

&lt;210&gt; 65

&lt;211&gt; 170

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 65

Met	Gln	Ser	Lys	Ile	Leu	Ser	Phe	Val	Leu	Phe	Thr	Leu	Ser	Leu	Gly
1															

Tyr	Val	Leu	Gly	Glu	Thr	Cys	Ser	Asn	Ala	Lys	Val	Lys	Gly	Ala	Thr
20															

Ser	Tyr	Ser	Thr	Thr	Asp	Ala	Thr	Ile	Val	Ser	Gln	Ile	Ala	Phe	Val
35															

Thr	Glu	Phe	Ser	Leu	Glu	Cys	Ser	Asn	Pro	Gly	Ser	Glu	Lys	Ile	Ser
50															

Leu Phe Ala Glu Val Asp Gly Lys Ile Thr Pro Val Ala Met Ile Gly  
 65 70 75 80

Asp Thr Thr Tyr Gln Val Ser Trp Asn Glu Glu Val Asn Lys Ala Arg  
 85 90 95

Ser Gly Asp Tyr Ser Val Lys Leu Tyr Asp Glu Glu Gly Tyr Gly Ala  
 100 105 110

Val Arg Lys Ala Gln Arg Ser Gly Glu Glu Asn Lys Val Lys Pro Leu  
 115 120 125

Ala Thr Val Val Val Arg His Pro Gly Thr Tyr Thr Gly Pro Trp Phe  
 130 135 140

Asn Ser Glu Ile Leu Ala Ala Gly Leu Ile Ala Val Val Ala Tyr Phe  
 145 150 155 160

Ala Phe Ser Thr Arg Ser Lys Ile Leu Ser  
 165 170

<210> 66  
 <211> 712  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 66  
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 gaaaacatgct caaatgctaa ggttaaggga gttacctttt attccacaac ggatgccaca 180  
 attgtaagcc aaattgcctt tggactgaa ttctccttgg aatgctaaaa tcctggatcc 240  
 gagaaaaatct ccctatttgc tgaagtcgat ggcaaaatta ctcctgttgc catgatcg 300  
 gataccacctt accaggtgag ctggaatgaa gaggttaata aggctagaag tggtgactac 360  
 agtgtgaagc tggactgatga agaaggatac ggagcgttac gcaaaagctca gagatcagg 420  
 gaagagaaca aggtcaaacc actagcaacc gttgttggt gacatccagg aacatacact 480  
 ggaccatgg tcaattccga aatcctcgca gctggtctca ttgctgttgc tgcctacttt 540  
 gctttctcaa cgcaagcaa aattcttcc taaagagacg cagcatgaaa tttcacaaaa 600  
 aaataaaaaac aaattcaagt catcaaccat gtctcttgg cactcagact gtttctgtga 660  
 aatacaaaact attatttaac aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa 712

<210> 67  
 <211> 73

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 67

Met	Val	Ser	Ile	Leu	Leu	Ile	Ser	Leu	Ile	Leu	Asn	Leu	Leu	Val	Phe
1				5				10						15	

Tyr	Ala	Lys	Ala	Arg	Pro	Leu	Glu	Asp	Ile	Ser	Ser	Asp	Leu	Ser	Pro
						20		25					30		

Asp	Tyr	Tyr	Ile	Thr	Glu	Gly	Tyr	Asp	Gly	Val	Lys	Glu	Lys	Arg	Glu
							35	40			45				

Ile	Glu	Leu	Val	Pro	Val	Thr	Phe	Gly	Ile	Phe	Asn	Ile	His	Thr	Thr
						50		55			60				

Pro	Ala	Pro	Arg	Ile	Thr	Phe	Glu	Trp
						65		70

&lt;210&gt; 68

&lt;211&gt; 379

&lt;212&gt; DNA

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 68

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gttggttttc	tatgctaaag	ctagaccact	agaagacatc	tcgtcagatc	tttccctgta	120
ttattacatc	actgaaggct	atgacggtgt	gaaggagaag	agagagatcg	aacttgtacc	180
tgtgacattt	ggaatattta	atatacatac	aacacctgct	cccagaatta	ccttgaatg	240
gtaaaaaaaaatc	caagaagaat	ttatgatttt	atttttcctt	ccattggat	ggattgtaag	300
tcagcataaaa	acgccgttaa	aaatgaattt	ttaataaaaaa	aaaattattc	caaaaaaaaaaa	360
aaaaaaaaaaaa	aaaaaaaaaa					379

&lt;210&gt; 69

&lt;211&gt; 76

&lt;212&gt; PRT

&lt;213&gt; Lutzomyia longipalpis

&lt;400&gt; 69

Met	Lys	Leu	Phe	Cys	Leu	Ile	Phe	Val	Val	Phe	Val	Ala	Leu	Glu	Val
1								10					15		

Cys	Ile	Glu	Thr	Val	Lys	Ala	Met	Glu	Ala	Thr	Glu	Glu	Ile	Ser	Val
						20		25					30		

Lys Leu Gln Asp Asp Ala Asn Glu Pro Asp Asp Ser Leu Asp Leu Asp  
 35 40 45

Glu Gly Leu Pro Asp Ala Phe Asp Glu Asp Tyr Asn Asn Gln Ala Glu  
 50 55 60

Tyr Lys Pro Asn Pro Arg Gly Asp Tyr Arg Arg Arg  
 65 70 75

<210> 70  
 <211> 526  
 <212> DNA  
 <213> Lutzomyia longipalpis

<400> 70  
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 tatctgtaaa attgcaagat gatgcgaatg aacctgatga ctctctggat ttagacgaag 180  
 gtcttcctga tgcattcgat gaggactata ataatcaggc tgagtacaag ccgaatccta 240  
 gaggggacta cagaagacga taattaatat aaattcagga aaacactcta aaaatttcca 300  
 attgactcta ctttaaacga tttataacctt acctacacta aataccatat gcaataatta 360  
 tgttttaatt atttagtgca agatctacta gtttcagttc atattttggg actttccgc 420  
 ctttctctcg atggaaaaat gattttacgg attcttaatt ttcattgtac agagttata 480  
 aaacaattga aagcaattaa aaaaaaaaaa aaaaaaaaaa aaaaaa 526

<210> 71  
 <211> 22  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Oligonucleotide primer

<400> 71  
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<210> 72  
 <211> 22  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Oligonucleotide primer

<400> 72  
 ctcttcgcta ttacgcccagc tg 22

<210> 73  
<211> 24  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Oligonucleotide primer

<400> 73  
tctcgggaag cgcgccattg tgtt

24