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(54) Title: HETEROLOGOUS FUSION PROTEIN CONSTRUCTS COMPRISING A *LEISHMANIA* ANTIGEN

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DPV
DPV-AC
MAPS-DPV
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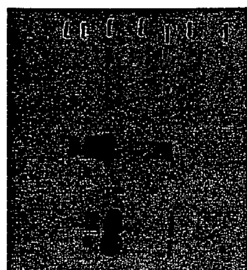

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DPV
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(57) Abstract: The present invention provides a recombinant nucleic acid molecule encoding a fusion polypeptide, wherein the recombinant nucleic acid comprises a heterologous polynucleotide sequence encoding an antigen or an antigenic fragment, and a *Leishmania* polynucleotide sequence encoding a polypeptide or fragment thereof, wherein the *Leishmania* polynucleotide is selected from the group consisting of TSA polynucleotide, LeIF polynucleotide, M15 polynucleotide, and 6H polynucleotide. The invention also provides an expression cassette comprising the recombinant nucleic acid molecule, host cells comprising the expression cassette, compositions, fusion polypeptides, and methods of their use in diagnosis or in generating a protective immune response in hosts.

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HETEROLOGOUS FUSION PROTEIN CONSTRUCTS COMPRISING A *LEISHMANIA* ANTIGEN

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CROSS-REFERENCES TO RELATED APPLICATIONS

The present application claims priority to USSN 60/275,837, filed March 13, 2001, herein incorporated by reference in its entirety.

The present application is related to U.S. patent application No. 09/056,556, 10 filed April 7, 1998; U.S. patent application No. 09/223,040, filed December 30, 1998; U.S. patent application No. 09/287,849, filed April 7, 1999; published PCT application No. WO 99/51748, filed April 7, 1999 (PCT/US99/07717); U.S. patent application No. 60/158,338, filed October 7, 1999; U.S. patent application No. 60/158,425, filed October 7, 1999; U.S. patent application No. 09/597,796, filed June 20, 2000; U.S. patent application No. 15 09/688,672, filed October 10, 2000; published PCT application No. WO 01/24820, filed October 10, 2000 (PCT/US00/28095); U.S. patent application No. 60/265,737, filed February 1, 2001; U.S. patent application No. 09/886,349, filed June 20, 2001; and published PCT application No. WO 01/98460, filed June 20, 2001 (PCT/US01/19959), and USSN 60/_______, filed February 15, 2002, TTC attorney docket number 014058-009080US, 20 herein each incorporated by reference in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

Not applicable.

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FIELD OF THE INVENTION

This present invention relates to recombinant nucleic acids containing *Leishmania* TSA, LeIF, M15 or 6H polynucleotide encoding a polypeptide or a fragment thereof and a heterologous polynucleotide encoding an antigen or an antigenic fragment, such as *Mycobacterium* sp. antigens. In particular, it relates to using these nucleic acids as DNA 30 vaccines to elicit protective immunity against pathogenic microorganisms in the host. The present invention also relates to expression cassettes comprising the recombinant nucleic

acids, host cells comprising the expression cassettes, compositions, fusion polypeptides, and methods of their use in diagnosis or in generating a protective immune response in hosts.

BACKGROUND OF THE INVENTION

Tuberculosis is a chronic infectious disease caused by infection with *M. tuberculosis* and other *Mycobacterium* species. It is a major disease in developing countries, as well as an increasing problem in developed areas of the world, with about 8 million new cases and 3 million deaths each year. Although the infection may be asymptomatic for a considerable period of time, the disease is most commonly manifested as an acute inflammation of the lungs, resulting in fever and a nonproductive cough. If untreated, serious complications and death typically result.

Although tuberculosis can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease. Infected individuals may be asymptomatic, but contagious, for some time. In addition, although compliance with the treatment regimen is critical, patient behavior is difficult to monitor. Some patients do not complete the course of treatment, which can lead to ineffective treatment and the development of drug resistance.

In order to control the spread of tuberculosis, effective vaccination and accurate early diagnosis of the disease are of utmost importance. Currently, vaccination with live bacteria is the most efficient method for inducing protective immunity. The most common *Mycobacterium* employed for this purpose is *Bacillus Calmette-Guerin* (BCG), an avirulent strain of *M. bovis*. However, the safety and efficacy of BCG is a source of controversy and some countries, such as the United States, do not vaccinate the general public with this agent.

Diagnosis of tuberculosis is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, which indicates exposure to *Mycobacterium* antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

While macrophages have been shown to act as the principal effectors of *Mycobacterium* immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against *Mycobacterium* infection is illustrated by the frequent occurrence of *Mycobacterium* infection in AIDS patients, due to the depletion of

$CD4^+$ T cells associated with human immunodeficiency virus (HIV) infection. *Mycobacterium*-reactive $CD4^+$ T cells have been shown to be potent producers of γ-interferon (IFN-γ), which, in turn, has been shown to trigger the anti-mycobacterial effects of macrophages in mice. While the role of IFN-γ in humans is less clear, studies have shown that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-γ or tumor necrosis factor-alpha, activates human macrophages to inhibit *M. tuberculosis* infection. Furthermore, it is known that IFN-γ stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, interleukin-12 (IL-12) has been shown to play a role in stimulating resistance to *M. tuberculosis* infection. For a review of the immunology of *M. tuberculosis* infection, *see* Chan & Kaufmann, *Tuberculosis: Pathogenesis, Protection and Control* (Bloom ed., 1994), and *Harrison's Principles of Internal Medicine*, volume 1, pp. 1004-1014 and 1019-1023 (14th ed., Fauci *et al.*, eds., 1998).

Accordingly, there is a need for improved diagnostic reagents, and improved methods for diagnosis, preventing and treating tuberculosis. Embodiments of the invention meet this and other goals.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that when a heterologous polynucleotide sequence is fused to a *Leishmania* thiol-specific thiol-specific-antioxidant (herein referred to as "TSA" or "MAPS"), the *Leishmania* polynucleotide increases the expression of heterologous polynucleotide in eukaryotic cells. In addition to *Leishmania* TSA polynucleotide, embodiments of the invention provide that other *Leishmania* polynucleotides that expresses at a high level in eukaryotic cells, such as LeIF (a *L. braziliensis* gene homologous to the eukaryotic ribosomal protein eIF4A, also referred to as "LbeIF4A"), M15 (L. major stress-inducible 1 or LmSTI1) or 6H (*L. braziliensis* gene homologous to the gene for the eukaryotic 83-kDa heat shock protein, also referred to as "Lbhsp83"), can also be used to make fusion constructs of the invention. Embodiments of the invention also provide that by optimizing the codons of the heterologous polynucleotides for maximal expression in eukaryotic cells, the expression of the fusion constructs can be further enhanced in eukaryotic cells. The *Leishmania* antigen can be at the N- or C-terminal region of the fusion protein, or may be found at any position in a fusion protein that comprises more than two antigens.

Any suitable heterologous polynucleotides that encode an antigen or an antigenic fragment can be fused to the *Leishmania* TSA, LeIF, M15 or 6H sequences.

Typically, the heterologous polynucleotide is selected from those that encode a viral antigen such as HIV, HSV, CMV, or an Ebola antigen, a malaria antigen, a cancer antigen, or a bacterial antigen. In a preferred embodiment, a heterologous polynucleotide is a *Mycobacterium* polynucleotide sequence encoding an antigen or antigenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex. In another preferred embodiment, the antigen is a *Mycobacterium* fusion protein, e.g., MTB72F ± 85b antigen. In another embodiment, the fusion protein comprises an RA35 antigen (full length, mature, or N-terminal portion of mature or full length Ra35) with a serine to alanine mutation at the triad active site at amino acid position 183 in wild-type MTB32A (Ra35).

The present fusion constructs are useful for enhancing the expression of *Mycobacterium* polynucleotides, as well as other heterologous polynucleotides which otherwise express poorly in eukaryotic cells. Moreover, the present invention constructs are particularly useful, among others, as DNA vaccines against, e.g., infections by one or more pathogenic microorganisms.

The present invention is also based, in part, on the discovery that when a heterologous polynucleotide is fused to a *Leishmania* TSA polynucleotide, the *Leishmania* polynucleotide fusion polypeptide elicits a strong cellular immune response when administered to a mammal. Thus, the present fusion constructs are useful, among others, in eliminating altered self-cells (e.g., virus-infected cells and tumor cells) in the host.

Accordingly, in one aspect, the invention provides a recombinant nucleic acid molecule encoding a fusion polypeptide, wherein the recombinant nucleic acid comprises a heterologous polynucleotide encoding an antigen or an antigenic fragment, and a *Leishmania* polynucleotide sequence encoding a polypeptide or a fragment thereof, wherein the *Leishmania* polynucleotide is selected from the group consisting of TSA polynucleotide, LeIF polynucleotide, M15 polynucleotide, and 6H polynucleotide. The invention also provides an expression cassette comprising the recombinant nucleic acid molecule, host cells comprising the expression cassette, and compositions comprising the expression cassette, and fusion polypeptides.

In one embodiment, the fusion polynucleotide and polypeptide comprise a relatively short fragment of a gene or a polypeptide, respectively, derived from the *Leishmania* TSA, LeIF, M15 or 6H so that a minimal immune response is elicited against the *Leishmania* polypeptide fragment in the host. In another embodiment, the heterologous polynucleotide is a *Mycobacterium* polynucleotide, preferably those that encode for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB12 antigen, MTB32A antigen, MTB40

antigen, MTB41 antigen, TbH9 antigen, Ra35 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85 complex antigen, or an immunogenic fragment thereof. In another embodiment, *Mycobacterium* polynucleotide encodes for fusion proteins with two or
5 more *Mycobacterium* antigens, such as MTB59F antigen, MTB72F antigen, MTB31F antigen, MTB71F antigen, or an immunogenic fragment thereof. In another embodiment, the *Mycobacterium* polynucleotide is codon optimized for expression in eukaryotic cells.

In another aspect, recombinant nucleic acid molecules, expression cassettes, compositions and fusion polypeptides may be used as immunogens to generate or elicit a
10 protective immune response in a patient. The polynucleotides may be administered directly into a subject as DNA vaccines to cause antigen expression in the subject, and the subsequent induction of, e.g., an anti-*M. tuberculosis* immune response. Alternatively, the isolated or purified polynucleotides are used to produce recombinant fusion polypeptide antigens *in vitro*, which are then administered as a vaccine. Thus, the isolated or purified fusion
15 *Leishmania* polypeptides and nucleic acids of the invention may be formulated as pharmaceutical compositions for administration into a subject in the prevention or treatment of *Leishmania* infections and/or infections by other microorganisms, such as *M. tuberculosis*. The immunogenicity of the fusion protein or antigens may be enhanced by the inclusion of an adjuvant, as well as additional fusion polypeptides, from *Mycobacterium* or other organisms,
20 such as bacterial, viral, mammalian polypeptides. Additional polypeptides may also be included in the compositions, either linked or unlinked to the fusion polypeptide or compositions.

In another aspect, recombinant nucleic acid molecules, expression cassettes, compositions and fusion polypeptides of the invention are used in *in vitro* and *in vivo* assays
25 for detecting humoral antibodies or cell-mediated immunity against one or more pathogenic microorganisms (e.g., *M. tuberculosis* and/or *Leishmania*) for diagnosis of infection or monitoring of disease progression. For example, the polypeptides may be used as an *in vivo* diagnostic agent in the form of an intradermal skin test. The polypeptides may also be used in *in vitro* tests such as an ELISA with patient serum. Alternatively, the nucleic acids, the
30 compositions, and the fusion polypeptides may be used to raise, e.g., anti-*M. tuberculosis* antibodies in a non-human animal. The antibodies can be used to detect the target antigens *in vivo* and *in vitro*.

DEFINITIONS

"*Leishmania* polynucleotide" that encodes a polypeptide or a fragment thereof refers to a native *Leishmania* polynucleotide found in *Leishmania* cells, fragments thereof, or any conservatively modified variants thereof. Functionally, a *Leishmania* polynucleotide has the ability to produce a fusion protein, and enhances expression relative to expression of a native full length *Mycobacterium* polynucleotide or portion thereof, or fusion thereof (e.g., MTB8.4, MTB12, MTB72F, 85b complex antigen, MTB72F plus 85b complex antigen (MTB103F), TB38-1 antigen, etc.) by at least 10%, optionally at least by 20%, 30%, 40%, 50%, 100%, or 200%.

"Fusion polypeptide" or "fusion protein" refers to a protein having at least two heterologous polypeptides covalently linked, either directly or via an amino acid linker. The polypeptides forming the fusion protein are typically linked C-terminus to N-terminus, although they can also be linked C-terminus to C-terminus, N-terminus to N-terminus, or N-terminus to C-terminus. The polypeptides of the fusion protein can be in any order. This term also refers to conservatively modified variants, polymorphic variants, alleles, mutants, subsequences, and interspecies homologs of the antigens that make up the fusion protein. In embodiments of the invention, typically a *Leishmania* polypeptide or a fragment thereof is fused to a heterologous polypeptide, such as *Mycobacterium tuberculosis* antigen or a fragment thereof. The *Leishmania* antigen can be fused to the *Mycobacterium tuberculosis* antigen (or other heterologous antigen) at either the N-or C-terminus, or for a fusion protein of more than two members, at any position. *Mycobacterium tuberculosis* antigens are described in Cole *et al., Nature* 393:537 (1998), which discloses the entire *Mycobacterium tuberculosis* genome. The complete sequence of *Mycobacterium tuberculosis* can also be found at http://www.sanger.ac.uk and at http://www.pasteur.fr/mycdb/ (MycDB). Antigens from other *Mycobacterium* species that correspond to *M. tuberculosis* antigens can be identified, e.g., using sequence comparison algorithms, as described herein, or other methods known to those of skill in the art, e.g., hybridization assays and antibody binding assays.

Typically, a fusion polypeptide of the invention specifically binds to antibodies raised against at least two antigen polypeptides, wherein each antigen polypeptide is selected from the group consisting of a *Leishmania* TSA, LeIF, M15 or 6H polypeptide and a heterologous polypeptide, such as a *Mycobacterium* polypeptide. The antibodies can be polyclonal or monoclonal. Optionally, the fusion polypeptide specifically binds to antibodies raised against the fusion junction of the antigens, which antibodies do not bind to the antigens

individually, i.e., when they are not part of a fusion protein. The fusion polypeptides optionally comprise additional polypeptides, e.g., three, four, five, six, or more polypeptides, up to about 25 polypeptides, optionally heterologous polypeptides or repeated homologous polypeptides, fused to the at least two heterologous antigens. The additional polypeptides of
5 the fusion protein are optionally derived from *Mycobacterium* as well as other sources, such as other bacterial, viral, or invertebrate, vertebrate, or mammalian sources. The individual polypeptides of the fusion protein can be in any order. As described herein, the fusion protein can also be linked to other molecules, including additional polypeptides. The compositions of the invention can also comprise additional polypeptides that are unlinked to
10 the fusion proteins of the invention. These additional polypeptides may be heterologous or homologous polypeptides.

The term "fused" refers to the covalent linkage between two polypeptides in a fusion protein. The polypeptides are typically joined via a peptide bond, either directly to each other or via an amino acid linker. Optionally, the peptides can be joined via non-peptide
15 covalent linkages known to those of skill in the art.

"FL" refers to full-length, i.e., a polypeptide that is the same length as the wild-type polypeptide.

In some instances in the application, Ra35 refers to the N-terminus of MTB32A (Ra35FL), comprising at least about the first 205 amino acids of MTB32A from *M.*
20 *tuberculosis*, or the corresponding region from another *Mycobacterium* species. Ra12 refers to the C-terminus of MTB32A (Ra35FL), comprising at least about the last 132 amino acids from MTB32A from *M. tuberculosis*, or the corresponding region from another *Mycobacterium* species.

The following provides sequences of some individual antigens used in the
25 compositions and fusion proteins of the invention:

MTB32A (TbRa35FL), the sequence of which is disclosed as SEQ ID NO:17 (cDNA) and SEQ ID NO:79 (protein) in the U.S. patent applications No. 08/523,436, 08/523,435, No. 08/658,800, No. 08/659,683, No. 08/818,112, No. 09/056,556, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications, see also Skeiky et al.,
30 Infection and Immunity 67:3998-4007 (1999);

MTBRa12, the C-terminus of MTB32A (Ra35FL), comprising at least about the last 132 amino acids from MTB32A from M. tuberculosis, the sequence of which is disclosed as SEQ ID NO:4 (DNA) and SEQ ID NO:66 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967;

Ra35, the N-terminus of MTB32A (Ra35FL), comprising at least about the first 205 amino acids of MTB32A from M. tuberculosis, the nucleotide and amino acid sequence of which is disclosed in Figure 4;

MTB39 (TbH9), the sequence of which is disclosed as SEQ ID NO:106
5 (cDNA full length) and SEQ ID NO:107 (protein full length) in the U.S. patent applications No. 08/658,800, No. 08/659,683, No. 08/818,112, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications. The sequence is also disclosed as SEQ ID NO:33 (DNA) and SEQ ID NO:91 (amino acid) in U.S. patent application No. 09/056,559;

The following provides sequences of some fusion proteins of the invention
10 TbH9-Ra35 (MTB59F), the sequence of which is disclosed as SEQ ID NO:23 (cDNA) and SEQ ID NO:24 (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application;

RA12-TbH9-Ra35 (MTB72F), the sequence of which is disclosed as SEQ ID NO:1 (DNA) and SEQ ID NO:2 (protein) in the US patent application No. 09/223,040, No.
15 09/223,040, and in the PCT/US99/07717 application.

RA12-TbH9-Ra35-85b antigen (MTB103F), the sequence of which is disclosed in USSN 60/____, filed February 15, 2002, TTC reference no. 014058-009080US.

The following provides sequences of some additional antigens used in the compositions and fusion proteins of the invention:
20 MTB8.4 (DPV), the sequence of which is disclosed as SEQ ID NO:101 (cDNA) and SEQ ID NO:102 (protein) in the U.S. patent applications No. 08/658,800, No. 08/659,683, No. 08/818,112 and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications;

MTB9.8 (MSL), the sequence of which is disclosed as SEQ ID NO:12 (DNA),
25 SEQ ID NO:109 (predicted amino acid sequence) and SEQ ID NO:110 to 124 (peptides) in the U.S. patent applications No. 08/859,381, No. 08/858,998, No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications;

MTB9.9A (MTI, also known as MTI-A), the sequence of which is disclosed as SEQ ID NO:3 and SEQ ID NO:4 (DNA) and SEQ ID NO:29 and SEQ ID NO:51 to 66 (ORF
30 peptide for MTI) in the U.S. patent applications No. 08/859,381, No. 08/858,998, No. 09/073,009 and v09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications. Two other MTI variants also exist, called MTI-B and MTI-C;

MTB40 (HTCC#1), the sequence of which is disclosed as SEQ ID NO:137 (cDNA) and 138 (predicted amino acid sequence) in the U.S. patent applications No.

09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications;

MTB41 (MTCC#2), the sequence of which is disclosed as SEQ ID NO:140 (cDNA) and SEQ ID NO:142 (predicted amino acid sequence) in the U.S. patent applications No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications;

ESAT-6, the sequence of which is disclosed as SEQ ID NO:103 (DNA) and SEQ ID NO:104 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967. The sequence of ESAT-6 is also disclosed in U.S. Patent No. 5,955,077.

TB38-1, the sequence of which is disclosed as SEQ ID NO:46 (DNA) and SEQ ID NO:88 (amino acid) in USSN 08/818,112 and USSN 09/072,967.

α-crystalline antigen, the sequence of which is disclosed in Verbon *et al., J. Bact.* 174:1352-1359 (1992);

85 complex antigen, e.g., 85b complex antigen, the sequence of which is disclosed in Content *et al., Infect. & Immunol.* 59:3205-3212 (1991).

Each of the above sequences is also disclosed in Cole *et al. Nature* 393:537 (1998) and can be found at, e.g., http://www.sanger.ac.uk and http:/www.pasteur.fr/mycdb/.

The above sequences are disclosed in U.S. patent applications Nos. 08/523,435, 08/523,436, 08/658,800, 08/659,683, 08/818,111, 08/818,112, 08/942,341, 08/942,578, 08/858,998, 08/859,381, 09/056,556, 09/072,596, 09/072,967, 09/073,009, 09/073,010, 09/223,040, 09/287,849 and in PCT patent applications PCT/US98/10407, PCT/US98/10514, PCT/US99/03265, PCT/US99/03268, PCT/US99/07717, WO97/09428 and WO97/09429, WO98/16645, WO98/16646, each of which is herein incorporated by reference.

MTB32AMutSA is a mutated version of wild-type MTB32A (Ra35FL or Ra35 mature). The sequence of wild-type RA35 is disclosed as SEQ ID NO:17 (cDNA) and SEQ ID NO:79 (protein) in the U.S. patent applications No. 08/523,436, 08/523,435, No. 08/658,800, No. 08/659,683, No. 08/818,112, No. 09/056,556, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications, *see also* Skeiky *et al., Infection and Immunity* 67:3998-4007 (1999). The term mutated MTB32, mutated MTB32A, MTB32AMutSA or MTB32MutSA includes MTB32A amino acid sequences in which any one of the three amino acids at the active site triad (His, Asp, Ser, amino acid positions 182-184 of the wild type molecule), e.g., the serine residue at amino acid position 183 in wild-type MTB32A, has been

changed to another amino acid (e.g., to alanine, Ra35FLMutSA, *see, e.g.*, the sequence comparison of wild type and mutated MTB32 in Figure 5).

The term "immunogenic fragment thereof" refers to a polypeptide comprising an epitope that is recognized by cytotoxic T lymphocytes, helper T lymphocytes or B cells.

5 The term "*Mycobacterium* species of the tuberculosis complex" includes those species traditionally considered as causing the disease tuberculosis, as well as *Mycobacterium* environmental and opportunistic species that cause tuberculosis and lung disease in immune compromised patients, such as patients with AIDS, e.g., *M. tuberculosis, M. bovis,* or *M. africanum, BCG, M. avium, M. intracellulare, M. celatum, M. genavense, M. haemophilum,* 10 *M. kansasii, M. simiae, M. vaccae, M. fortuitum,* and *M. scrofulaceum (see, e.g., Harrison's Principles of Internal Medicine*, volume 1, pp. 1004-1014 and 1019-1023 (14th ed., Fauci *et al.*, eds., 1998).

An adjuvant refers to the components in a vaccine or therapeutic composition that increase the specific immune response to the antigen (*see, e.g.*, Edelman, *AIDS Res. Hum* 15 *Retroviruses* 8:1409-1411 (1992)). Adjuvants induce immune responses of the Th1-type and Th-2 type response. Th1-type cytokines (e.g., IFN-γ, IL-2, and IL-12) tend to favor the induction of cell-mediated immune response to an administered antigen, while Th-2 type cytokines (e.g., IL-4, IL-5, Il-6, IL-10 and TNF-β) tend to favor the induction of humoral immune responses.

20 "Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the 25 reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) 30 and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka *et al.*, *J.*

Biol. Chem. 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

As used herein, the terms "DNA segment" and "polynucleotide" refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a polypeptide refers to a DNA segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the terms "DNA segment" and "polynucleotide" are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

The terms "isolated," "purified," or "biologically pure" therefore refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Of course, this refers to the DNA segment as originally isolated, and does not exclude other isolated proteins, genes, or coding regions later added to the composition by the hand of man. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. An isolated nucleic acid is separated from other open reading frames that flank the gene and encode proteins other than the gene.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

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

in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein, which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally

identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

(*see, e.g.*, Creighton, *Proteins* (1984)).

The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (e.g., a fusion protein).

The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

An "expression cassette" refers to a polynucleotide molecule comprising expression control sequences operatively linked to coding sequence(s).

A "vector" is a replicon in which another polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment.

"Antibody" refers to a polypeptide comprising a framework region from an immunoglobulin gene or fragments thereof that specifically binds and recognizes an antigen.
5 The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

10 An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and
15 variable heavy chain (V_H) refer to these light and heavy chains respectively.

Antibodies exist, e.g., as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The $F(ab)'_2$
20 may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the $F(ab)'_2$ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see Fundamental Immunology* (Paul ed., 3d ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either
25 chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990)).

30 For preparation of monoclonal or polyclonal antibodies, any technique known in the art can be used (*see, e.g.*, Kohler & Milstein, *Nature* 256:495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pp. 77-96 in *Monoclonal Antibodies and Cancer Therapy* (1985)). Techniques for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce antibodies to polypeptides of this invention.

Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990); Marks *et al.*, *Biotechnology* 10:779-783 (1992)).

The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to fusion proteins can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with fusion protein and not with individual components of the fusion proteins. This selection may be achieved by subtracting out antibodies that cross-react with the individual antigens. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an individual antigen or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not diminished, relative to a fusion polypeptide comprising native antigens. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native polypeptide or a portion thereof.

The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the

same (i.e., 70% identity, optionally 75%, 80%, 85%, 90%, or 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the compliment of a test sequence. Optionally, the identity exists over a region that is at least about 25 to about 50 amino acids or nucleotides in length, or optionally over a region that is 75-100 amino acids or nucleotides in length.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 25 to 500, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (*see, e.g., Current Protocols in Molecular Biology* (Ausubel *et al.*, eds. 1995 supplement)).

Another example of algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al., Nuc. Acids Res.* 25:3389-3402 (1977) and Altschul *et al., J. Mol. Biol.* 215:403-410 (1990), respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information

(http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-C illustrate Western blots of DNA vaccine construct expression in HEK 293 cells. Panel A. Rabbit anti-DPV: MAPS-DPV-AC is strongly expressed in HEK cells, while all other DPV constructs are undetectable. Panel B. Rabbit anti-DPAS: While all

DPAS constructs are expressed, fusion with MAPS results in increased expression. Panel C. Rabbit anti-MAPS: This panel demonstrates that, of the DPV constructs, only MAPS-DPV-AC is expressed and that MAPS-DPAS and MAPS-DPAS-AC are expressed at comparable levels. JA4304, negative control, shows no reactivity with any antibody.

5 Figures 2 illustrates Western blots of DNA vaccine construct expression in HEK 293 cells. The left panel shows reactivity of fusion proteins with rabbit anti-DPV. The right panel shows reactivity of fusion proteins with rabbit anti-MAPS. Data indicate that fusion of the codon optimized DPV gene to sequences encoding the first give (MAPS(N5)/DPV-AC) and, in particular, the first ten (MAPS(N10)/DPV-AC) amino acids of
10 MAPS significantly boosts the expression of these antigens in eukaryotic cells. The full length MAPS/DPV-AC are most highly expressed.

Figures 3A and 3B illustrate nucleotide and amino acid sequences of *Leishmania* thiol-specific-antioxidant (i.e., TSA or MAPS) having SEQ ID NOS: 66 and 67, respectively.

15 Figure 4 illustrate nucleotide and amino acid sequences of *Leishmania* LeIF (i.e., LbeIF4A) having SEQ ID NOS: 68 and 69, respectively.

Figure 5 illustrate nucleic acid and amino acid sequences of *Leishmania* M15 (i.e., LmSTI1) having SEQ ID NOS: 70 and 71, respectively.

Figure 6 illustrate nucleic acid and amino acid sequences of *Leishmania* 6H
20 (i.e., Lbhsp83) having SEQ ID NOS: 72 and 73, respectively.

DETAILED DESCRIPTION

INTRODUCTION

Vaccination with antigen encoding DNA constructs is an attractive alternative to protein-based vaccines. One potential problem for DNA vaccination, however, is that the
25 level of antigen expression sufficient to elicit protective immunity is often not achieved. In some situations, this may be due to the fact that non-secreted, intracellular, heterologous proteins may not be highly expressed in eukaryotic cells. In other situations, this may be due to the fact that many organisms utilize codons differentially to obtain optimum protein expression. Therefore, a gene derived from an infectious disease agent, containing that
30 microorganism's inherent codon bias, may not be expressed at a level high enough to provide protection in a mammalian model system of the disease. For example, low protein expression occurs for some *Mycobacterium tuberculosis* genes tested in DNA vaccination studies.

The present inventors discovered that the fusion to a gene known to express at high levels in eukaryotic cells can enhance the expression of heterologous polynucleotides in eukaryotic cells. For example, many *Leishmania* genes express at a high level in eukaryotic cells. These genes include, e.g., thiol-specific-antioxidant (herein referred to as "TSA" or "MAPS," *see, e.g.*, Webb *et al., Infection and Immunity* 66:3279-3289 (1998)), LeIF (also referred to as "LbeIF4A," see, e.g., Skeiky et al., J. Exp. Med. 181:1527-1537 (1995); Skeiky *et al., J. Immunol.* 161:6171-6179 (1998)), M15 (also referred to as "LmSTI1," *see, e.g.*, Webb *et al., J. Immunol.* 157:5034-5041 (1996)), and 6H (also referred to as "Lbhsp83," *see, e.g.*, Skeiky *et al., Infection and Immunity* 63:4105-4114 (1995)). Preferably, these *Leishmania* sequences are fused at the N-terminus of the heterologous polynucleotide to enhance the efficiency of ribosome movement and hence the translation efficiency of the mRNA. In a preferred embodiment, TSA is used to produce a fusion construct. Moreover, the expression of a heterologous polynucleotide fused to these *Leishmania* polynucleotide can be further enhanced by optimizing the codon usage of the heterologous polynucleotide for maximal expression in eukaryotic cells. Therefore, the present fusion constructs are particularly useful as DNA vaccines to prevent, e.g., infections by pathogenic microorganisms.

Any heterologous sequences of interest can be fused to the *Leishmania* TSA, LeIF, M15 or 6H sequences. These include, but are not limited to, a *Mycobacterium* antigen, a HIV antigen, a HSV antigen, a CMV antigen, a malaria antigen, a cancer antigen, or other viral or bacterial antigens. Expression of these heterologous sequences can be enhanced by fusing them to the above described *Leishmania* sequences. Moreover, it has been found that the fusion of a heterologous sequence to a *Leishmania* sequence can elicit a strong cellular immune response in mammalian host. Thus, the present fusion constructs are useful, among others, in eliminating altered self-cells (e.g., virus-infected cells and tumor cells) in the host.

Accordingly, the present invention provides recombinant nucleic acid molecules encoding a fusion polypeptide, wherein the nucleic acid molecule comprises a heterologous polynucleotide sequence of interest and a *Leishmania* polynucleotide encoding a polypeptide or a fragment thereof, wherein the *Leishmania* polynucleotide is TSA polynucleotide, LeIF polynucleotide, M15 polynucleotide, or 6H polynucleotide. The invention also provides expression cassettes comprising the recombinant nucleic acid molecules, compositions comprising the expression cassettes, fusion polypeptides, and methods for their use.

Embodiments of the invention have many applications. For example, the present invention can be used to produce DNA vaccines against infections by microorganisms, such as *Mycobacterium*. In another example, by fusing polynucleotides that encode epitopes from two or more microorganisms, the present invention can be used as a vaccine against diseases caused by different infectious agents (e.g., *Mycobacterium* and *Leishmania*). In another example, embodiments of the invention can be used *in vitro* and *in vivo* assays for detecting humoral antibodies or cell-mediated immunity against *Mycobacterium* or other microorganisms for diagnosis of infection or monitoring of disease progression. Embodiments of the invention and their use are described in detail below.

RECOMBINANT NUCLEIC ACID MOLECULES

In one aspect, the invention provides recombinant nucleic acid molecules comprising a *Leishmania* TSA, LeIF, M15 or 6H polynucleotide sequence encoding a polypeptide or fragment thereof and a polynucleotide encoding an antigen or antigenic fragment of a microorganism, such as *Mycobacterium*. Recombinant nucleic acids are constructed so that, preferably, the *Leishmania* polynucleotide is located 5' to a heterologous polynucleotide sequence of interest. It may also be appropriate to place a *Leishmania* polynucleotide 3' to the heterologous polynucleotide sequence or to insert the heterologous polynucleotide sequence into a site within the *Leishmania* polynucleotide.

The recombinant nucleic acid molecules of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention. Preferably, the recombinant sequences are operably linked to a eukaryotic promoter, such as CMV, to provide expression cassettes.

1. LEISHMANIA POLYNUCLEOTIDES

Any suitable *Leishmania* polynucleotides can be used for constructing recombinant fusion nucleic acid molecules of the present invention. For example, the *Leishmania* polynucleotides can be derived from TSA gene (*see* Webb *et al., Infect. Immun.* 66:3279-3289 (1998); GenBank Accession No. AF044679), LeIF gene (Skeiky *et al., J. Exp. Med.* 181:1527-1537 (1995); Skeiky *et al., J. Immunol.* 161:6171-6179 (1998)), M15 gene (Webb *et al., J. Immunol.* 157:5034-5041 (1996); GenBank Accession No. 473845), or 6H gene (Skeiky *et al., J. Infec. Immun.* 63:4105-4114 (1995)), all of the disclosures of which are incorporated herein by reference. The nucleic acid and amino acid sequences of LeIF, M15 and 6H are also described in U.S. Patent No. 5,834,592, incorporated herein by reference. These genes express highly in eukaryotic cells, and any of these *Leishmania* genes can be used to make a fusion polynucleotide.

Typically, fusion to these *Leishmania* polynucleotides increase the expression of a heterologous polynucleotide fused to these *Leishmania* polynucleotides by at least 10%, optionally at least 20%, 30%, 40%, 50%, 100% or 200%, compared to the expression of the heterologous polynucleotide alone.

Either the full length *Leishmania* gene or a portion of the *Leishmania* gene can be included in fusion polynucleotides of the invention. For example, the *Leishmania* polynucleotides that encode a polypeptide or a fragment thereof can comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths," in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

The selection of the length and portion of the *Leishmania* polynucleotide depends on whether an immune response against the *Leishmania* polypeptide is desired. If it is desired to elicit an immune response against a *Leishmania* polypeptide portion of the fusion construct, then the full length *Leishmania* gene or a portion that encodes a highly antigenic epitope is used. These constructs are capable of serving as an effective vaccine against at least two different infectious agent (*Leishmania* and another microorganism from which the fusion partner is derived).

If minimizing an immune response against a *Leishmania* polypeptide is desired, then preferably small fragments of a *Leishmania* gene are used. For example, a *Leishmania* polynucleotide included in the fusion construct may comprise about 90 nucleotides or less, about 60 nucleotides or less, about 30 nucleotides or less, about 15 nucleotides or less, or any intermediate lengths in between. Preferably, a *Leishmania* polynucleotide includes at least the 5' portion of a *Leishmania* gene. As described in the example section, a *Leishmania* polynucleotide comprising the first 15 nucleotides or the first 30 nucleotides of the *Leishmania* TSA gene can enhance the expression of its fusion partner, without eliciting much immune response to TSA.

In embodiments of the invention, *Leishmania* polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes TSA, LeIF, M15, 6H or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not diminished, relative to a fusion polypeptide comprising a native *Leishmania* polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native *Leishmania* polynucleotide or a portion thereof. Optionally, the identity exists over a region that is at least about 25 to about 50 nucleotides in length, or optionally over a region that is 75-100 nucleotides in length. Variants are preferably capable of hybridizing under stringent conditions to the native *Leishmania* sequences.

2. FUSION PARTNERS TO LEISHMANIA POLYNUCLEOTIDES

In the present invention, any suitable heterologous polynucleotides of interest can be selected as a fusion partner to *Leishmania* polynucleotides. Typically, heterologous polynucleotides encode pathogenic antigens, bacterial antigens, viral antigens, cancer antigens, tumor antigens, tumor suppressors, or antigenic fragments thereof. In one embodiment, heterologous polynucleotides encode an antigen or antigenic fragment from a *Mycobacterium* species of the tuberculosis complex. In another embodiment, heterologous polynucleotides are derived from infectious agents, such as HIV, HSV, CMR, Ebola, or pathogenic agents that cause malaria (e.g., *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*).

Preferably, the fusion partner is derived from *Mycobacterium* polynucleotides encoding *Mycobacterium* antigens or fragments thereof can be coupled to a *Leishmania*

polynucleotide. *Mycobacterium* polynucleotides are derived from a *Mycobacterium* species of the tuberculosis complex, e.g., a species such as *M. tuberculosis, M. bovis,* or *M. africanum,* or a *Mycobacterium* species that is environmental or opportunistic and that causes opportunistic infections such as lung infections in immune compromised hosts (e.g., patients
5 with AIDS), e.g., *BCG, M. avium, M. intracellulare, M. celatum, M. genavense, M. haemophilum, M. kansasii, M. simiae, M. vaccae, M. fortuitum,* and *M. scrofulaceum (see, e.g., Harrison's Principles of Internal Medicine*, volume 1, pp. 1004-1014 and 1019-1023 (14th ed., Fauci *et al.*, eds., 1998).

In embodiments of the invention, *Mycobacterium* polynucleotides can encode
10 a single antigen or immunogenic fragments thereof, or can encode at least two heterologous *Mycobacterium* antigens or immunogenic fragments thereof. Some fusion proteins comprising at least two heterologous *Mycobacterium* antigens, or immunogenic fragments thereof are sometimes highly antigenic. The antigens of the present invention may further comprise other components designed to enhance the antigenicity of the antigens or to
15 improve these antigens in other aspects, for example, the isolation of these antigens through addition of a stretch of histidine residues at one end of the antigen.

Examples of *Mycobacterium* polynucleotides that can be fused to a *Leishmania* polynucleotide include those that encode *Mycobacterium* sp. antigens such as MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB12 antigen, MTB32A antigen,
20 MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4 antigen, DPPD antigen, MTB92 antigen, Erd14 antigen, ESAT-6 antigen, MTB85 complex antigen, MTB59F antigen, MTB72F antigen, MTB31F antigen, MTB71 antigen, or immunogenic fragment thereof.

The heterologous polynucleotide which is linked to a *Leishmania*
25 polynucleotide encodes a polypeptide or a fragment comprising at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53,
30 *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

In embodiments of the invention, heterologous polynucleotides may comprise a native sequence (e.g., an endogenous sequence from an organism's cells) or may comprise a conservatively modified variant of such a sequence or immunogenic fragment thereof.

Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the biological activity of the encoded fusion polypeptide is not diminished, relative to a fusion polypeptide comprising a native heterologous polypeptide. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native polynucleotide or a portion thereof. Optionally, the identity exists over a region that is at least about 25 to about 50 nucleotides in length, or optionally over a region that is 75-100 nucleotides in length. Variants are preferably capable of hybridizing under stringent conditions to the native sequences.

In some embodiments, the heterologous polynucleotides are optimized for eukaryotic codon selection, particularly for human and/or primate. As described above, most organisms exhibit differential codon usage for optimum protein expression. Thus, the expression of a *Mycobacterium* sp. genes in eukaryotic cells is often very poor. The expression of *Mycobacterium* or other heterologous polynucleotides can be enhanced by optimizing the codon usage of the polynucleotides for maximal expression in eukaryotic cells. Preferably, the codons are optimized for expression in mammals, particularly in human and/or in primates. The preferred codon usage in mammals and other vertebrates are described in, e.g., *Current Protocols in Molecular Biology*, vol. 4, Ausubel et al., ed., John Wiley & Sons, Inc., Appendix 1, incorporated herein by reference. Codon usage tables can also be found in www.kazusa.or.jp/codon/, incorporated herein by reference.

The following provides sequences of some *Mycobacterium* sp. antigens used in embodiments of the invention:

SEQ ID NO:1-4: MTB32A (Ra35FL or Ra35 mature), the sequence of which is also disclosed as SEQ ID NO:17 (cDNA) and SEQ ID NO:79 (protein) in the U.S. patent applications No. 08/523,436, 08/523,435, No. 08/658,800, No. 08/659,683, No. 08/818,112, No. 09/056,556, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications, *see also* Skeiky *et al., Infection and Immunity* 67:3998-4007 (1999). The term MTB32A also includes MTB32A amino acid sequences in which any one of the three amino acids at the active site triad (His, Asp, Ser), e.g., the serine residue at amino acid position 207 in SEQ ID NO:2 or amino acid position 183 in SEQ ID NO:4, has been changed to another amino acid (e.g., alanine, Ra35FLMutSA, *see, e.g.*, Figure 6 and SEQ ID NO:6).

SEQ ID NO:5 and 6: Ra35FLMut SA, the mature version of RA35FL in which the serine residue at amino acid position 183 of SEQ ID NO:4 has been changed to an alanine residue.

SEQ ID NO:7 and 8: Ra35, the N-terminus of MTB32A (Ra35FL), comprising at least about 195 amino acids from the N-terminus of MTB32A from *M. tuberculosis*, the nucleotide and amino acid sequence of which is disclosed in Figure 4 (*see also* amino acids 33-227 of SEQ ID NO:2 and amino acids 8 to 202 of SEQ ID NO:4). The term Ra35 (N-term) also includes Ra35 amino acid sequences in which any one of the three amino acids at the active site triad (i.e., His, Asp, or Ser) has been changed as described above.

SEQ ID NO:9 and 10: MTBRa12, the C-terminus of MTB32A (Ra35FL), comprising at least about 132 amino acids from the C-terminus of MTB32A from *M. tuberculosis* (*see, e.g.*, amino acids 224 to 355 of SEQ ID NO:2 and amino acids 199 to 330 of SEQ ID NO:4), the sequence of which is disclosed as SEQ ID NO:4 (DNA) and SEQ ID NO:66 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967.

SEQ ID NO:11, 12, 13, and 14: MTB39 (TbH9), the sequence of which is disclosed as SEQ ID NO:106 (cDNA full length) and SEQ ID NO:107 (protein full length) in the U.S. patent applications No. 08/658,800, No. 08/659,683, No. 08/818,112, and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications. The sequence is also disclosed as SEQ ID NO:33 (DNA) and SEQ ID NO:91 (amino acid) in U.S. patent application No. 09/056,559.

The following provides sequences of some fusion *Mycobacterium* sp. proteins of the invention

SEQ ID NO:15 and 16: MTB72F (Ra12-TbH9-Ra35), the sequence of which is disclosed as SEQ ID NO:1 (DNA) and SEQ ID NO:2 (protein) in the US patent application No. 09/223,040, No. 09/223,040, and in the PCT/US99/07717 application. The term MTB372F also includes MTB72F amino acid sequences in which any one of the three amino acids at the active site triad in Ra35FL (i.e., His, Asp, or Ser), has been changed as described above (*see, e.g.*, MTB72FMutSA, Figure 5).

SEQ ID NO:17 and 18: MTB72FMutSA (Ra12-TbH9-Ra35MutSA), wherein, in the Ra35 component of the fusion protein, the serine at position 710 has been changed to an alanine.

SEQ ID NO:19 and 20: TbH9-Ra35 (MTB59F), the sequence of which is disclosed as SEQ ID NO:23 (cDNA) and SEQ ID NO:24 (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application.

The following provides sequences of some additional antigens used in the compositions and fusion proteins of the invention:

SEQ ID NO: 21 and 22: MTB8.4 (DPV), the sequence of which is disclosed as SEQ ID NO:101 (cDNA) and SEQ ID NO:102 (protein) in the U.S. patent applications No. 08/658,800, No. 08/659,683, No. 08/818,112 and No. 08/818,111 and in the WO97/09428 and WO97/09429 applications.

SEQ ID NO:23 and 24: MTB9.8 (MSL), the sequence of which is disclosed as SEQ ID NO:12 (DNA), SEQ ID NO:109 (predicted amino acid sequence) and SEQ ID NO:110 to 124 (peptides) in the U.S. patent applications No. 08/859,381, No. 08/858,998, No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications.

SEQ ID NO:25, 26, and 27: MTB9.9A (MTI, also known as MTI-A), the sequence of which is disclosed as SEQ ID NO:3 and SEQ ID NO:4 (DNA) and SEQ ID NO:29 and SEQ ID NO:51 to 66 (ORF peptide for MTI) in the U.S. patent applications No. 08/859,381, No. 08/858,998, No. 09/073,009 and v09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications. Two other MTI variants also exist, called MTI-B and MTI-C.

SEQ ID NO:28 and 29: MTB40 (HTCC#1), the sequence of which is disclosed as SEQ ID NO:137 (cDNA) and 138 (predicted amino acid sequence) in the U.S. patent applications No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications.

SEQ ID NO:30 and 31: MTB41 (MTCC#2), the sequence of which is disclosed as SEQ ID NO:140 (cDNA) and SEQ ID NO:142 (predicted amino acid sequence) in the U.S. patent applications No. 09/073,009 and No. 09/073,010 and in the PCT/US98/10407 and PCT/US98/10514 applications.

SEQ ID NO:32 and 33: ESAT-6, the sequence of which is disclosed as SEQ ID NO:103 (DNA) and SEQ ID NO:104 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967. The sequence of ESAT-6 is also disclosed in U.S. Patent No. 5,955,077.

SEQ ID NO:34 and 35: Tb38-1 or 38-1 (MTb11), the sequence of which is disclosed in SEQ ID NO:46 (DNA) and SEQ ID NO:88 (predicted amino acid) in the U.S. patent application Nos. 09/072,96; 08/523,436; 08/523,435; 08/818,112; and 08/818,111; and in the WO97/09428 and WO97/09429 applications.

SEQ ID NO:36 and 37: TbRa3, the sequence of which is disclosed in SEQ ID NO:15 (DNA) and SEQ ID NO:77 (predicted amino acid sequence) of WO 97/09428 and WO97/09429 applications.

SEQ ID NO:38 and 39: 38 kD, the sequence of which is disclosed in SEQ ID
5 NO:154 (DNA) and SEQ ID NO:155 (predicted amino acid sequence) in the U.S. patent application No. 09/072,967. 38 kD has two alternative forms, with and without the N-terminal cysteine residue.

SEQ ID NO:40 and 41: DPEP, the sequence of which is disclosed in SEQ ID NO:52 (DNA) and SEQ ID NO:53 (predicted amino acid sequence) in the WO97/09428 and
10 WO97/09429 publications.

SEQ ID NO:42 and 43: TbH4, the sequence of which is disclosed as SEQ ID NO:43 (DNA) and SEQ ID NO:81 (predicted amino acid sequence) in WO97/09428 and WO97/09429 publications.

SEQ ID NO:44 and 45: DPPD, the sequence of which is disclosed in SEQ ID
15 NO:240 (DNA) and SEQ ID NO:241 (predicted amino acid sequence) in USSN 09/072,967 and in the PCT/US99/03268 and PCT/US99/03265 applications. The secreted form of DPPD is shown herein in Figure 12 of PCT/US00/28095.

MTb82 (MTb867), the sequence of which is disclosed in Figures 8 (DNA) and 9 (amino acid) of PCT/US00/2809.

20 Erd14 (MTb16), the cDNA and amino acids sequences of which are disclosed in Verbon *et al., J. Bacteriology* 174:1352-1359 (1992).

α-crystalline antigen, the sequence of which is disclosed in Verbon *et al., J. Bact.* 174:1352-1359 (1992);

85 complex antigen, the sequence of which is disclosed in Content *et al.,*
25 *Infect. & Immunol.* 59:3205-3212 (1991).

The following provides sequences of some additional fusion proteins used in the compositions and fusion proteins of the invention:

SEQ ID NO:46 and 47: DPV-MTI-MSL-MTCC#2 (MTb71F), the sequence
30 of which is disclosed as SEQ ID NO:15 (nucleic acid) and in SEQ ID NO:16: (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application.

SEQ ID NO:48 and 49: DPV-MTI-MSL (MTb31F), the sequence of which is disclosed in SEQ ID NO:18 (cDNA) and SEQ ID NO:19 (protein) in the U.S. patent application No. 09/287,849 and in the PCT/US99/07717 application.

Each of the above sequences is also disclosed in Cole *et al. Nature* 393:537 (1998) and can be found at, e.g., http://www.sanger.ac.uk and http:/www.pasteur.fr/mycdb/.

The above sequences are disclosed in U.S. patent applications Nos.
5 08/523,435, 08/523,436, 08/658,800, 08/659,683, 08/818,111, 08/818,112, 08/942,341, 08/942,578, 08/858,998, 08/859,381, 09/056,556, 09/072,596, 09/072,967, 09/073,009, 09/073,010, 09/223,040, 09/287,849 09/597,796; and in PCT patent applications PCT/US00/28095; PCT/US98/10407, PCT/US98/10514, PCT/US99/03265, PCT/US99/03268, PCT/US99/07717, WO97/09428 and WO97/09429, WO98/16645,
10 WO98/16646, each of which is herein incorporated by reference.

In the nomenclature of the application, Ra35 refers to the N-terminus of MTB32A (Ra35FL), comprising at least about 195 to 205 amino acids of MTB32A from *M. tuberculosis*, or the corresponding region from another *Mycobacterium* species. Ra12 refers to the C-terminus of MTB32A (Ra35FL), comprising at least about the last 132 amino acids
15 from MTB32A from *M. tuberculosis*, or the corresponding region from another *Mycobacterium* species.

3. EXAMPLES OF FUSION BETWEEN LEISHMANIA AND MYCOBACTERIUM SEQUENCES

The following provides sequences of fusion nucleic acid constructs between a
20 *Leishmania* TSA polynucleotide and a *Mycobacterium* sp. polynucleotide, and proteins encoded by the fusion polynucleotides:

SEQ ID NO:50 and 51: MAPS-DPVpET is a fusion DNA construct comprising *Leishmania* gene TSA at the N-terminus and linked with the TB antigen DPV (aka MTB8.4). SEQ ID NO:50 is a nucleotide sequence and SEQ ID NO:51 is the
25 corresponding amino acid sequence. This construct is used for protein expression.

SEQ ID NO:52 and 53: MAPS-DPASpET is a fusion DNA construct comprising *Leishmania* gene TSA at the N-terminus and linked with the TB antigen DPAS (aka MTB12). SEQ ID NO:52 is a nucleotide sequence and SEQ ID NO:53 is the corresponding amino acid sequence. This construct is used for protein expression.

30 SEQ ID NO:54: MAPS-DPVpc is a fusion DNA vaccine construct comprising *Leishmania* gene TSA at the N-terminus and linked with the TB antigen DPV (aka MTB8.4).

SEQ ID NO:55: MAPS-DPASpc is a fusion DNA vaccine construct comprising *Leishmania* gene TSA at the N-terminus and linked with the TB antigen DPAS (aka MTB12).

SEQ ID NO:56 and 57: MAPS-DPV-AC is a fusion construct comprising *Leishmania* TSA at the N-terminus and linked with the TB antigen DPV (aka MTB8.4) which is codon optimized for expression in eukaryotic cells. SEQ ID NO:56 is a nucleotide sequence, and SEQ ID NO:57 is the corresponding amino acid sequence.

SEQ ID NO:58 and 59: MAPS-DPAS-AC is a fusion construct comprising *Leishmania* TSA at the N-terminus and linked with the TB antigen DPAS (aka MTB12) which is codon optimized for expression in eukaryotic cells. SEQ ID NO:58 is a nucleotide sequence, and SEQ ID NO:59 is the corresponding amino acid sequence.

SEQ ID NO:60 and 61: MAPS(N5)-DPV-AC is a fusion construct comprising the first five amino acids of *Leishmania* TSA at the N-terminus and linked with the TB antigen DPV (aka MTB8.4) which is codon optimized for expression in eukaryotic cells. SEQ ID NO:60 is a nucleotide sequence, and SEQ ID NO:61 is the corresponding amino acid sequence.

SEQ ID NO:62 and 63: MAPS(N10)-DPV-AC is a fusion construct comprising the first ten amino acids of *Leishmania* TSA at the N-terminus and linked with the TB antigen DPV (aka MTB8.4) which is codon optimized for expression in eukaryotic cells. SEQ ID NO:62 is a nucleotide sequence, and SEQ ID NO:63 is the corresponding amino acid sequence.

SEQ ID NO:64 and 65: MTB72F-MAPS (aka r95f) is a fusion construct comprising a MTB72F (a 72 kDa poly-protein fusion construct comprising Ra12-TbH9-Ra35) linked to the *Leishmania* TSA. SEQ ID NO:64 is a nucleotide sequence, and SEQ ID NO:65 is the corresponding amino acid sequence.

These fusion constructs are merely exemplary, and one of skill in the art would readily recognize that any suitable *Leishmania* sequences can be linked to any other heterologous sequences of interest, particularly those derived from pathogenic agents.

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

The above-described polynucleotides may be identified, prepared and/or manipulated using any of a variety of well established techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (i.e., expression that is at least two

fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena *et al.*, *Proc. Natl. Acad. Sci. USA* 93:10614-10619 (1996) and Heller *et al.*, *Proc. Natl. Acad. Sci. USA* 94:2150-2155 (1997)). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as *M. tuberculosis or Leishmania* cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (e.g., a *M. tuberculosis* cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (1989)). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length,

have a GC content of at least 50% and anneal to the target sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia *et al.*, *Nucl. Acids Res.* 16:8186 (1988)), which uses restriction enzymes to generate a fragment in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom *et al.*, *PCR Methods Applic.* 1:111-19 (1991)) and walking PCR (Parker *et al.*, *Nucl. Acids. Res.* 19:3055-60 (1991)). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (e.g., NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, *Leishmania* fusion constructs may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-

naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

5 Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides

10 may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein.

15 For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

20 Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (*see* Caruthers, M. H. *et al., Nucl. Acids Res. Symp. Ser.* pp. 215-223 (1980), Horn *et al., Nucl. Acids Res. Symp. Ser.* pp. 225-232 (1980)). Alternatively, the protein itself may be produced using chemical methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example,

25 peptide synthesis can be performed using various solid-phase techniques (Roberge *et al., Science* 269:202-204 (1995)) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, *Proteins, Structures and Molecular*

30 *Principles* (1983)) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical

methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described in Sambrook *et al., Molecular Cloning, A Laboratory Manual* (1989), and Ausubel *et al., Current Protocols in Molecular Biology* (1989).

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high

level expression of fusion proteins that are readily purified may be used. Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the
5 subsequent 7 residues of β-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke &Schuster, *J. Biol. Chem.* 264:5503-5509 (1989)); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads
10 followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be
15 used. For reviews, see Ausubel *et al.* (*supra*) and Grant *et al.*, *Methods Enzymol.* 153:516-544 (1987).

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination
20 with the omega leader sequence from TMV (Takamatsu, *EMBO J.* 6:307-311 (1987)). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi *et al.*, *EMBO* J. 3:1671-1680 (1984); Broglie *et al., Science* 224:838-843 (1984); and Winter *et al., Results Probl. Cell Differ.* 17:85-105 (1991)). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-
25 mediated transfection. Such techniques are described in a number of generally available reviews (*see, e.g.*, Hobbs *in McGraw Hill Yearbook of Science and Technology* pp. 191-196 (1992)).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is
30 used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia larvae*. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene

inactive and produce recombinant virus lacking coat protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia larvae* in which the polypeptide of interest may be expressed (Engelhard *et al., Proc. Natl. Acad. Sci. U.S.A.* 91 :3224-3227 (1994)).

5 In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to
10 obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. U.S.A.* 81:3655-3659 (1984)). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient
15 translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational
20 control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those
25 described in the literature (Scharf. *et al., Results Probl. Cell Differ.* 20:125-162 (1994)).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational
30 processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al., Cell* 11:223-32 (1977)) and adenine phosphoribosyltransferase (Lowy *et al., Cell* 22:817-23 (1990)) genes which can be employed in tk^- or $aprt^-$ cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler *et al., Proc. Natl. Acad. Sci. U.S.A.* 77:3567-70 (1980)); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al., J. Mol. Biol.* 150:1-14 (1981)); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* 85:8047-51 (1988)). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al., Methods Mol. Biol.* 55:121-131 (1995)).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding

sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. 5 These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific 10 for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, 15 among other places, in Hampton *et al., Serological Methods, a Laboratory Manual* (1990) and Maddox *et al., J. Exp. Med.* 158:1211-1216 (1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to 20 polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. 25 These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be 30 cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the encoded polypeptide

through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-
5 tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen. San Diego, Calif.) between the purification domain and the encoded polypeptide may be used
10 to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath *et al., Prot. Exp. Purif.* 3:263-281 (1992) while the enterokinase cleavage site provides
15 a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll *et al., DNA Cell Biol.* 12:441-453 (1993)).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase
20 techniques (Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963)). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

25 ***IN VIVO* POLYNUCLEOTIDE DELIVERY TECHNIQUES**

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety or well known approaches, several of which are outlined below for the purpose of illustration.

30 *1. ADENOVIRUS*

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression

vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

5 The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus & Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA 10 can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

15 Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the 20 onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the 25 viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

30 In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process.

Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was
5 transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones & Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham & Prevec, 1991). In nature, adenovirus can package approximately 105% of the wild-type genome
10 (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of
15 the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or
20 epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, e.g., Vero cells or other monkey embryonic mesenchymal or epithelial cells. As stated above, the currently preferred helper cell line is 293.

Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293
25 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is
30 added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an

MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, e.g., 10^9-10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus & Horwitz, 1992; Graham & Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet & Perricaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993), peripheral intravenous injections (Herz & Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5′ and 3′ ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas & Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex

class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

AAV (Ridgeway, 1988; Hermonat & Muzycska, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka & McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs. There are two major genes in the AAV genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins, and transcription from p40 produces the capsid proteins (Hermonat & Muzyczka, 1984).

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

4. OTHER VIRAL VECTORS AS EXPRESSION CONSTRUCTS

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.*, 1990). This suggested that large portions of the genome could be replaced with foreign genetic material. The hepatotropism and persistence (integration) were particularly attractive properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal

segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

5 In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. 10 Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty & Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest 15 may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several 20 devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

Selected organs including the liver, skin, and muscle tissue of rats and mice 25 have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, i.e., *ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present invention.

POLYPEPTIDES

30 The present invention, in other aspects, provides fusion polypeptides and compositions comprising the fusion polypeptides. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species. Preferably, the polypeptide is encoded by a polynucleotide

sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid
5 sequence disclosed herein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As
10 used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (i.e., they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a *Mycobacterium* sp. protein is a portion that reacts with such antisera and/or T-cells at a level
15 that is not substantially less than the reactivity of the full length polypeptide (e.g., in an ELISA and/or T-cell reactivity assay). Similarly, an immunogenic portion of a *Leishmania* protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide. Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity
20 of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988). For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound
25 antibodies detected using, for example, ^{125}I-labeled Protein A.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate
30 host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or

polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

5 Polypeptides of the invention, immunogenic fragments thereof, and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis 10 method, where amino acids are sequentially added to a growing amino acid chain. *See* Merrifield, *J. Am. Chem. Soc.* 85:2149-2146 (1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

15 In embodiments of the invention, a fusion polypeptide comprises *Leishmania* TSA, LeIF, M15 or 6H polypeptide or a fragment thereof and a heterologous polypeptide. Any heterologous polypeptide of interest can be fused to the *Leishmania* polypeptide. Typically, the heterologous polypeptide is a HIV antigen, a malaria antigen, a cancer antigen, a viral antigen or a bacterial antigen. Preferably, the heterologous polypeptide is a 20 *Mycobacterium* antigen or an antigenic fragment thereof. For example, the fusion partner to the *Leishmania* polypeptide is selected from *Mycobacterium* antigens or antigenic fragments thereof, such as MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4 antigen, DPPD 25 antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85 complex antigen, MTB59F antigen, MTB72F antigen, MTB31F antigen, or MTB71F antigen.

Fusion polypeptides of the present invention can further comprise one or more additional polypeptides. For example, an additional fusion partner may assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized 30 by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to

desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

Within preferred embodiments, an additional immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza* B (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (e.g., the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells. Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemaglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the additional immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the LytA gene; *Gene* 43:265-292 (1986)). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology* 10:795-798 (1992)). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

Fusion polypeptides of the present invention may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This

permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46 (1985); Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262 (1986); U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a *Mycobacterium* antigen. Such cells may generally be prepared *in vitro* or *ex*

vivo, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; *see also* U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a polypeptide of the invention, a polynucleotide encoding such a polypeptide, and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, the polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a polypeptide of the invention if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* 54:1065-1070 (1994). Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a polypeptide of the invention (100 ng/ml - 100 μg/ml, preferably 200 ng/ml - 25 μg/ml) for 3-7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-γ) is indicative of T cell activation (*see* Coligan *et al.*, *Current Protocols in Immunology*, vol. 1 (1998)). T cells that have been activated in response to a polypeptide, polynucleotide or polypeptide-expressing APC may be $CD4^+$ and/or $CD8^+$. Protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, $CD4^+$ or $CD8^+$ T cells that proliferate in response to a polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize the polypeptide. Alternatively, one or more T cells that proliferate in the presence of the protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell and/or antibody compositions disclosed herein in pharmaceutically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA compositions that express a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, e.g., other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

1. ORAL DELIVERY

In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be

formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

The active compounds may even be incorporated with excipients and used in
5 the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as
10 dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be
15 present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially
20 non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the
25 weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing
30 such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For

example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or
5 added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

2. INJECTABLE DELIVERY

10 In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be
15 prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous
20 solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U. S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as
25 bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention
30 of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the

use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion (*see, e.g.*, *Remington's Pharmaceutical Sciences*, 15th Edition, pp. 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily

administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

3. NASAL DELIVERY

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described e.g., in U. S. Patent 5,756,353 and U. S. Patent 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U. S. Patent 5,780,045 (specifically incorporated herein by reference in its entirety).

4. LIPOSOME-, NANOCAPSULE-, AND MICROPARTICLE-MEDIATED DELIVERY

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In

particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon & Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, 1990; Muller *et al.*, 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath & Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta & Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller & Baltimore, 1984), transcription factors and allosteric effectors (Nicolau & Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trails examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori & Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm.

Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, i.e. in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but

nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for h or days, depending on their composition, and half lives in the blood range from min to several h. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to
5 intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be are easily made, as described (Couvreur *et al.,* 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684,
10 specifically incorporated herein by reference in its entirety).

VACCINES

In certain preferred embodiments of the present invention, vaccines are provided. As shown in Example 5 below, the present fusion constructs elicit a strong cell-mediated immune response. The cell-mediated immune system responds to endogenous
15 antigen presented by the MHC class I processing pathway. Cells can process foreign proteins found in the cell cytosol and display relevant peptide epitopes using this processing pathway (Harding, in *Cellular Proteolytic Systems*, pp. 163-180 (1994); Carbone & Bevan, in *Fundamental Immunology*, pp. 541-567 (Paul, ed., 1989); Townsend & Bodmer, *Annu. Rev. Immunol.* 7: 601-624 (1989)). The MHC class I processing pathway involves
20 digestion of the antigen by the proteasome complex and transport of the resulting peptides into the endoplasmic reticulum, where they bind to nascent MHC class I molecules (Germain & Margulies, *Annu. Rev. Immunol.* 11: 403-450 (1993)). Cytotoxic T lymphocytes (CTLs) specifically recognize the foreign antigen displayed by the MHC class I molecules and lyse the antigen-presenting cells. A population of memory T cells is also
25 established that can react to presentation of the specific antigen. The cellular immune system is thus primed to swiftly respond to an intracellular infection by a pathogenic organism such as a virus. The objective for a vaccine that stimulates the cell-mediated immune system is to deliver protein antigen to the cell cytosol for processing and subsequent presentation by MHC class I molecules.
30 The "MHC class I processing pathway" is an intracellular pathway that results in the binding of a peptide antigen ligand to an MHC class I molecule and the presentation of the antigen-MHC class I complex on the cell surface. First, cytoplasmic antigen is partially

processed (through the action of proteasomes) and enters the ER as a complex with a transporter protein. In the ER, MHC class I molecules stably associate with the peptide antigen. The antigen-MHC class I complex then passes through the trans-Golgi network in a secretory vesicle to the cell surface. Functionally, processing of a peptide antigen through the
5 MHC class I processing pathway can be identified with the use of lactacystin. Lactacystin is a specific proteasome inhibitor. Lactacystin inhibition of antigen presentation demonstrates that processing of the antigen is dependent on the function of the proteasome complex rather than an alternative processing pathway.

The present vaccines will generally comprise one or more pharmaceutical
10 compositions, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (e.g., polylactic galactide) and liposomes (into which the compound is incorporated; *see, e.g.,* Fullerton, U.S. Patent No. 4,235,877).
15 Vaccine preparation is generally described in, for example, Powell & Newman, eds., *Vaccine Design* (the subunit and adjuvant approach) (1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a
20 separate compound, within the composition or vaccine.

Illustrative vaccines may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral
25 expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198 (1998), and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as
30 *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch *et al.*, *Proc.*

Natl. Acad. Sci. USA 86:317-321 (1989); Flexner *et al., Ann. N.Y. Acad. Sci.* 569:86-103 (1989); Flexner *et al., Vaccine* 8:17-21 (1990); U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627 (1988); Rosenfeld *et al., Science* 252:431-434 (1991); Kolls *et al., Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Kass-Eisler *et al., Proc. Natl. Acad. Sci. USA* 90:11498-11502 (1993); Guzman *et al., Circulation* 88:2838-2848 (1993); and Guzman *et al., Cir. Res.* 73:1202-1207 (1993). Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al., Science* 259:1745-1749 (1993) and reviewed by Cohen, *Science* 259:1691-1692 (1993). The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a vaccine may comprise both a polynucleotide and a polypeptide component. Such vaccines may provide for an enhanced immune response.

It will be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the vaccine compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252. One may also employ a carrier comprising the particulate-protein complexes described in U.S. Patent

No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium* species or *Mycobacterium* derived proteins. For example, delipidated, deglycolipidated *M. vaccae* ("pVac") can be used. In another embodiment, BCG is used as an adjuvant. In addition, the vaccine can be administered to a subject previously exposed to BCG. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 and derivatives thereof (SmithKline Beecham, Philadelphia, PA); CWS, TDM, Leif, aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (e.g., IFN-γ, TNFα, IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines

will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann & Coffman, *Ann. Rev. Immunol.* 7:145-173 (1989).

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; *see* US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352 (1996). Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins. Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β-escin, or digitonin.

Alternatively, the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, *etc.* The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamelar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol^R to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one preferred embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. Another particularly

preferred adjuvant formulation employing QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and
5 QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (e.g., SBAS-2, AS2', AS2,'' SBAS-4, or SBAS6, available from SmithKline
10 Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

15 Other preferred adjuvants include adjuvant molecules of the general formula (I): $HO(CH_2CH_2O)_n$-A-R, wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein n is between 1 and 50,
20 preferably 4-24, most preferably 9; the R component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and A is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl
25 ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if
30 desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained

release formulation (i.e., a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes *et al.*, *Vaccine* 14:1429-1438 (1996)) and administered by, for example,
5 oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be
10 biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising
15 an amphiphilic compound, such as a phospholipid (*see, e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

20 Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the
25 capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (i.e., matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

30 Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau & Steinman, *Nature* 392:245-251 (1998)) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman & Levy, *Ann. Rev. Med.* 50:507-529 (1999)). In general, dendritic cells may be

identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up, process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly
5 found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel *et al.*, *Nature Med.* 4:594-600 (1998)).

Dendritic cells and progenitors may be obtained from peripheral blood, bone
10 marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNFα to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow
15 may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNFα, CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes.
20 However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fcγ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T
25 cell activation such as class I and class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a protein (or portion or other variant thereof) such that the polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a
30 composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally

be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi *et al.*, *Immunology and Cell Biology* 75:456-460 (1997). Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to a protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a protein of the invention.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: DNA constructs Comprising the *Leishmania* gene TSA at the N-terminus and Linked with the TB Antigens, MTB8.4 or MTB12

The following DNA constructs comprising the *Leishmania* gene TSA (also referred to as "MAPS") at the N-terminus linked with the TB antigens (DPV & DPAS; aka Mtb8.4 and Mtb12) were produced. The DNA (genetic fusion construct) was cloned into the eukaryotic DNA expression vector (pcDNA3) for transfection and DNA vaccine studies. Specifically, the following nucleic acid constructs were made: 1) MAPS-DPV pET (same as TSA-Mtb8.4; SEQ ID NO:50); and 2) MAPS-DPAS pET (same as TSA-Mtb12; SEQ ID NO:52). These constructs are for protein expression for the generation of recombinant antigens. Also made are the following constructs: 3) MAPS-DPV pcDNA (same as TSA-Mtb8.4; SEQ ID NO:54); and 4) MAPS-DPAS pcDNA (same as TSA-Mtb12; SEQ ID NO:55). These constructs are useful as DNA vaccine constructs. The protein sequences are also provided: 1) MAPS-DPV pET.pro (SEQ ID NO:51); and 2)MAPS-DPAS-pET.pro (SEQ ID NO:53). These sequences are recombinant proteins expressed from the corresponding nucleotide sequences described above with 6xHis residues for purification.

Example 2: DNA constructs Comprising the *Leishmania* gene TSA at the N-terminus and Linked with the Codon Optimized TB Antigens, MTB8.4 or MTB12

Highly expressed *Leishmania* TSA gene (also referred to as MAP) was fused with the codon optimized Mtb antigens DPV-AC (Mtb8.4) and DPAS-AC (Mtb12). The MAPS fusion vector was constructed in the plasmid pcDNA3.1 (Invitrogen). The MAPS

gene was amplified by PCR using primers that removed the MAPS termination codon and introduced an EcoRI cloning site at the 3' end of the coding sequence.

DPV-AC (altered codon) and DPAS-AC were constructed as follows. The coding sequences for the *M. tuberculosis* (Mtb) antigens DPV and DPAS were reconstructed to bias the codon usage toward that of mammalian, in this case murine, genes. To determine which codons of DPV and DPAS to alter a comparison was made of the Mtb and Mus musculus codon usage tables (www.kazusa.or.jp/codon/), which are based on the analysis of 1432158 and 4168443 codons, respectively. DPV and DPAS codons that are used infrequently in murine coding sequences were changed to the most frequently used Mus musculus codons. In order to avoid overrepresenting altered codons, in cases where a particular DPV or DPAS codon was repeatedly changed from low frequency Mtb usage to high frequency Mus musculus usage, the next most frequently used Mus musculus codon was substituted. This resulted in hypothetical altered codon DPV and DPAS coding sequences (DPV-AC and DPAS-AC) with a Mus musculus codon bias. DPV-AC and DPAS-AC were constructed using a series of codon biased sense and antisense oligonucleotides.

For DPV-AC 8 pairs of sense and antisense oligonucleotides, ranging in length from 28 bp to 40 bp were designed. Oligonucleotide pair 1, consisting of oligonucleotides 1 and 2, incorporated a HindIII site for subsequent cloning into vector JA4304, a Kozak consensus sequence and an ATG start codon 5' of the DPV-AC coding sequence. Oligonucleotide pair 8 included a NheI site for cloning into JA4304 3' of the TAA stop codon. Specifics for the individual DPV-AC oligonucleotides are as follows; DPV-AC oligo # position comments 1 sense (s) -19 / 18 D of DPV is +1 and G of ATG is –1. 2 antisense (as) -19 / 9 pair 1; 9 bp sense overhang. 3 s 19 / 56 4 as 10 / 46 pair 2; 9 bp as and 10 bp s overhang. 5 s 57 / 91 6 as 47 / 82 pair 3; 10 bp as and 9 bp s overhang. 7 s 92 / 127 8 as 83 / 118 pair 4; 9 bp as and 9 bp s overhang. 9 s 128 / 162 10 as 119 / 153 pair 5; 9 bp as and 9 bp s overhang. 11 s 163 / 199 12 as 154 / 190 pair 6; 9 bp as and 9 bp s overhang. 13 s 200 / 229 14 as 191 / 219 pair 7; 9 bp as and 10 bp s overhang. 15 s 230 / 249 includes 10 bp 3' of TAA containing NheI site. 16 as 220 / 249 pair 8; 10 bp as overhang.

For DPAS-AC 9 pairs of sense and antisense oligonucleotides, ranging in length from 33 bp to 47 bp were designed. Oligonucleotide pair 1, consisting of oligonucleotides 1 and 2, incorporated a HindIII site for subsequent cloning into vector JA4304, a Kozak consensus sequence and an ATG start codon 5' of the DPAS-AC coding sequence. Oligonucleotide pair 9 included a NheI site for cloning into JA4304 3' of the TGA stop codon. Specifics for the individual DPAS-AC oligonucleotides are as follows; DPAS-

AC oligo # position comments 1 sense (s) -19 / 24 D of DPAS is +1 and G of ATG is –1. 2 antisense (as) -19 / 14 pair 1; 10 bp sense overhang. 3 s 25 / 69 4 as 15 / 58 pair 2; 10 bp as and 11 bp s overhang. 5 s 70 / 115 6 as 59 / 106 pair 3; 11 bp as and 9 bps overhang. 7 s 116 / 160 8 as 107 / 150 pair 4; 9 bp as and 10 bp s overhang. 9 s 161 / 206 10 as 151 / 195 pair 5; 10 bp as and 11 bp s overhang. 11 s 207 / 251 12 as 196 / 241 pair 6; 11 bp as and 10 bp s overhang. 13 s 252 / 295 14 as 242 / 284 pair 7; 10 bp as and 11 bp s overhang. 15 s 296 / 340 16 as 285 / 327 pair 8; 11 bp as and 13 bp s overhang. 17 s 341 / 363 includes 10 bp 3' of TGA containing NheI site. 18 as 328 / 363 pair 9; 13 bp as overhang.

All DPV-AC and DPAS-AC oligonucleotides were obtained from Gibco-BRL. All oligonucleotides were reconstituted at 0.5 nmole/μl (~ 6 – 5 μg/μl) with H_2O. Pairs of oligonucleotides were combined (1 with 2, 3 with 4, etc., 17 with 18) in 20 μl annealing reactions containing 100 pmole/μl of each oligonucleotide in 10 mM Tris-HCl, pH 7.5, 0.1M NaCl, 10 mM EDTA. DPV-AC and DPAS-AC oligonucleotide pairs were placed at 65°C for 10 minutes and 94°C for 3 minutes, respectively, and allowed to anneal slowly at room temperature (25°C) for 90 minutes. DPV-AC and DPAS-AC oligonucleotide pairs were then diluted 20- and 10-fold with H_20 to 5 pmole/μl (~120 ng/μl) and 10 pmole/μl (~240ng/μl), respectively.

Next, 5 pmole and 10 pmole, respectively, of the individual DPV-AC and DPAS-AC oligonucleotide pairs were kinased with 10U of T4 polynucleotide kinase (Gibco-BRL) and 1 mM ATP for 10 minutes at 37°C. All DPV-AC or DPAS-AC oligonucleotide pairs were combined (~1 μg and 2 μg total DNA, respectively), placed at 65°C for 15 minutes to inactivate the kinase and then allowed to cool to room temperature for 30 minutes to anneal the overhangs. Ligations were performed by adding T4 DNA ligase reaction buffer to 1X to the annealed DPV-AC and DPAS-AC oligonucleotide pairs, adding 25U to 30U T4 DNA ligase (Gibco-BRL) and allowing the reactions to proceed for 3 hours at room temperature (25°C). Impurities were removed from the DPV-AC and DPAS-AC DNA using a Qiaquick gel extraction kit as per the manufacturers instructions (Qiagen).

The DPV-AC and DPAS-AC DNA was digested with HindIII and NheI, electrophoresed through 1.5% agarose and regions corresponding to the expected size products for DPV-AC and DPAS-AC (268 bp and 382 bp) were excised from the gel, isolated using a Qiaquick gel extraction kit and directionally cloned into JA4304. To confirm that DPV-AC and DPAS-AC in JA4304 were as expected the sense and antisense strands

were completely sequenced. The codon optimized DPAS-AC and DPV-AC DNA sequences are shown in SEQ ID NOS:66 and 67, respectively.

For fusion to MAPS, DPV-AC and DPAS-AC were PCR amplified with primers containing EcoRI restriction sites. The 5' primer EcoRI site allowed for DPV-AC
5 and DPAS-AC to be cloned in-frame to the 3' end of MAPS, while the 3' primer EcoRI site was placed downstream of a termination codon. Following PCR amplification, DPV-AC and DPAS-AC were gel purified, digested with EcoRI and cloned into EcoRI digested MAPS fusion vector. The resulting pcDNA3.1-based MAPS-DPV-AC (TSA-Mtb8.4-AC; SEQ ID NO:56) and MAPS-DPAS-AC (TSA-Mtb12-AC; SEQ ID NO:58) fusion plasmids were
10 verified by sequence analysis. The protein sequences of codon optimized MAPS-DPV-AC and MAPS-DPAS-AC are shown in SEQ ID NOS: 57 and 59, respectively.

Example 3: Protein Expression Levels of MAP-DPV-AC, MAP-DPAS-AC, MAP-DPV and MAP-DPAS, DPV and DPAS

The protein expression levels of the MAPS-DPV-AC and MAPS-DPAS-AC
15 were measured following their transfection into human embryonic kidney (HEK) 293 cells. These protein expression levels were compared to similarly transfected constructs encoding DPV, DPV-AC, MAPS-DPV and DPAS, DPAS-AC, MAPS-DPAS and empty JA4304. Briefly, about 2x 10^5 (~ 30% confluent) HEK 293 cells in DMEM/10% FBS were plated onto 35 mm culture dishes. DNA to be tested was brought to 1g in 10 L H_2O (0.1 g/l). The
20 FuGene 6 transfection reagent was prepared, and was added to the DNA. The FuGene 6/DNA mix was used to transfect the HEK 293 cells according to the manufacturer's instructions (Boehringer Mannheim). The HEK 293 cells were incubated for 48 to 72 hours at 37°C and harvested. The cells were collected by centrifugation for 7 minutes at 1.2 K rpm, resuspended in 250 L of 0.1M Tris, pH8, 4% SDS, 20% glycerol. After sonication for 30
25 seconds, the lysate protein concentration was determined by BCA assay (Pierce). 10 g of total protein was loaded per well, subjected to SDS PAGE and blotted to nitrocellulose. Rabbit polyclonal antibodies raised against DPV, DPAS and MAPS (1:10K dilution) followed by a donkey anti-rabbit HRP conjugated secondary antibody (1:10K dilution) and ECL (Amersham Pharmacia Biotech) were used to detect the expression of these proteins.
30 The lysates were also analyzed by SDS PAGE and coomassie staining.

Figures 1A-C illustrate Western blots of various DNA construct expression in HEK293 cells. As shown in the figures, data indicate that fusion of codon optimized DPV to MAPS (MAPS-DPV-AC) and fusion of DPAS to MAPS (MAPS-DPAS) significantly boosts

the expression of these antigens in eukaryotic cells. DPV is normally detectable on a Western blot only after a lengthy exposure and is never observed on a coomassie stained gel. The same holds true for DPV-AC and MAPS-DPV. Following fusion of MAPS to codon optimized DPV-AC, however, DPV expression is readily observed on a Western blot and is visible on SDS PAGE by coomassie staining. It is likely that the combination of DPV codon optimization and fusion to MAPS is required to achieve high level DPV expression, since neither DPV-AC nor MAPS-DPV (non-codon optimized) shows an increase in expression compared to the original DPV. In contrast to DPV, DPAS can be seen on Western blots and coomassie stained gels. When compared to DPAS and DPAS-AC, however, the MAPS-DPAS fusion results in a significant increase in the total amount of DPAS antibody reactive protein produced in the HEK 293 cells. This increase in protein expression appears dependent on fusion to MAPS, as the expression levels of DPAS-AC and MAPS-DPAS-AC are equivalent to the expression levels of DPAS and MAPS-DPAS, respectively.

Example 4: Truncated MAPS Constructs - MAPS(N5)/DPV-AC and MAPS(N10)/DPV-AC DNA and Their Expression

To minimize the immune response to MAPS while maintaining a high level of DPV expression, two truncated MAPS encoding the first five and ten amino acids of MAPS were fused to the DPV-AC gene. The constructs were evaluated for their ability to drive high level DPV expression in a DNA vaccine format. MAPS(N5)/DPV-AC (SEQ ID NOS:60 and 61) and MAPS(N10)/DPV-AC (SEQ ID NOS:62 and 63), hybrid sequences encoding DPV-AC downstream of the first five and ten amino of MAPS, respectively, were constructed in JA4304 as follows. The hybrid sequences were generated using the megaprimer PCR method. Primers MAPSN5-DPV-AC (5' GATAAAGCTTGCAATCATGTCCTGCGGT AACGACCCCGTGGACGCCGTGAT 3') and MAPSN10-DPV-AC (5'GATAAAGCTT GCAATCATGTCCTGCGGTAACGCCAAGATCAACTCTGACCCCGTGGACGCCGTGA T 3'), which include a HindIII restriction site, a kozak sequence, the coding sequence for the first five and ten amino acids of MAPS in frame with the sequence of DPV-AC, were used with primer DPV-AC-NheI-R (5' GATAGCTAGCTTAGTAGTTGTTGCAGGAGCCG 3') to amplify MAPS(N5)/DPV-AC and MAPS(N10)/DPV-AC. The PCR products were gel isolated, digested with HindIII and NheI, and cloned into HindIII/NheI cut JA4304. The inserts were fully sequenced to confirm that intact fusions were generated.

The protein expression levels of the MAPS(N5)/DPV-AC and MAPS(N10)/DPV-AC DNA vaccines were measured following their transfection into human

embryonic kidney (HEK) 293 cells and compared to similarly transfected constructs encoding DPV, DPV-AC, MAPS, MAPS/DPV, MAPS/DPV-AC and empty JA4304. Briefly, about 2 x 10^5 (~ 30% confluent) HEK 293 cells in DMEM/10% FBS were plated onto 35 mm culture dishes. DNA to be tested was brought to 1 g in 10 L H_2O (0.1 g/l). The
5 FuGene 6 transfection reagent was prepared, and was added to the DNA. The FuGene 6/DNA mix was used to transfect the HEK 293 cells according to the manufacturer's instructions (Boehringer Mannheim). The HEK 293 cells were incubated for 48 to 72 hours at 37°C and harvested. The cells were collected by centrifugation for 10 minutes at 1.2 K rpm, resuspended in 250 L PBS and lysed by the addition of 250 L of 0.1M Tris, pH 8, 4%
10 SDS, 20% glycerol. After sonication for 30 seconds, the lysate protein concentration was determined by BCA assay (Pierce). 10 g of total protein was loaded per well, subjected to SDS PAGE and blotted to nitrocellulose. Rabbit polyclonal antibodies raised against DPV and MAPS (1:10K dilution) followed by a donkey anti-rabbit HRP conjugated secondary antibody (1:10K dilution) and ECL (Amersham Pharmacia Biotech) were used to detect the
15 expression of these proteins. The lysates were also analyzed by SDS PAGE and coomassie staining.

Figure 2 illustrates Western blots of various DNA construct expression in HEK293 cells. As shown in the figure, data indicate that fusion of the codon optimized DPV gene to sequences encoding the first five (MAPS(N5)/DPV-AC) and, in particular, the first
20 ten (MAPS(N10)/DPV-AC) amino acids of MAPS significantly boosts the expression of these antigens in eukaryotic cells. DPV is normally detectable on a Western blot only after a lengthy exposure and is never observed on a coomassie stained gel. The same holds true for DPV-AC. Following the transfection of MAPS(N10)/DPV-AC into HEK 293 cells DPV expression is readily observed on a Western blot using rabbit anti DPV serum. DPV
25 expression is also elevated for MAPS(N5)/DPV-AC, although the level attained is much less than that seen for MAPS(N10)/DPV-AC. When the relative expression levels are compared, it is clear that fusions of DPV to full-length MAPS (MAPS/DPV and MAPS/DPV-AC) are most highly expressed, followed by the MAPS(N10)/DPV-AC fusion and then MAPS(N5)/DPV-AC.

30 It is important to note that all of the fusion constructs significantly elevate DPV expression compared to DPV alone. Interestingly, while full-length MAPS and its DPV hybrids react strongly with rabbit anti MAPS serum, MAPS(N10)/DPV-AC and MAPS(N5)/DPV-AC reactivity is barely detectable. This suggests that the first ten amino

acids of MAPS do not contain an antibody producing epitope. In summary, while MAPS(N10)/DPV-AC and MAPS(N5)/DPV-AC do not attain the extremely high levels of DPV expression observed for the fusion of full-length MAPS to DPV, they produce tremendous increases in the level of DPV expression, while generating virtually no MAPS
5 antibody response.

Example 5: Mouse Vaccination

The immune responses produced in mice by the various hybrid MAPS/DPV, MAPS and DPV DNA vaccines were compared. Groups of eight mice were immunized with 100 μg of the various MAPS/DPV DNA vaccines at three week intervals. Following the
10 immunizations, three of the mice were analyzed for a number of immune responses, including the production of IFN-γ, type-specific antibodies and cytotoxic T lymphocytes (CTL). Splenocytes from mice immunized with MAPS produced IFN-γ, only following restimulation with MAPS protein. In contrast, the splenocytes from MAPS/DPV immunized animals produced IFN-γ in response to MAPS and DPV recombinant proteins. Similarly,
15 while MAPS immunization produced IgG1 and IgG2a type-specific antibodies only to MAPS, immunization with MAPS/DPV resulted in the production of antibodies to both MAPS and DPV. Intriguingly, DPV DNA immunization alone produced no IFN-γ, or specific antibody, underscoring the ability of MAPS to increase DPV expression and subsequent immune responses.
20 Finally, the constructs were compared for their ability to generate DPV-specific CTL. DPV is known to generate CTL responses and did so in this experiment. A comparable level of CTL resulted from immunizations with MAPS(N10)/DPV. However, MAPS/DPV caused significantly higher levels of CTL production, suggesting that the increased expression of DPV driven by MAPS was responsible. In summary, these results
25 demonstrate that the fusion of MAPS to DPV does not diminish the ability of either antigen to stimulate the production of IFN-γ, antigen-specific antibodies or of DPV to mount a CTL response. Most notably, the high level expression of DPV results in an increased IFN-γ, type-specific antibody and CTL responses, suggesting that the MAPS/DPV hybrid DNA vaccine may prove more effective at protecting against tuberculosis infection.

Example 6: DNA constructs Comprising MTB72F/MAPS r95f

MTB72F has been shown to protect against TB challenge in three animal models (mouse, guinea pig and monkeys). Several other antigens shown to elicit T cell responses in healthy PPD positive donors are potential vaccine candidates. To improve on the protective efficacy of MTB72F, genetic fusion constructs with MTB72F as backbone were constructed. New MTB72F fusions MTB72F (a 72 kDa poly-protein fusion construct comprising Ra12-TbH9-Ra35) was used as a backbone to add several of other candidate antigens. These include, e.g., MTB72F/MAPS r95f. The nucleotide and polypeptide sequences of this construct are shown as SEQ ID NOS:64 and 65, respectively.

Example 7: DPV-AC-MAPs fusion protein with DPV-AC fused upstream of MAPS

A DPV-AC/MAPS DNA vaccine in JA4304 has been constructed for comparison to MAPS/DPV DNA vaccine in an effort to understand the mechanism underlying the ability of MAPS to boost DPV protein expression and DPV-specific immune responses.

MAPS (TSA) is a *Leishmania* major protein that is expressed at a high level from the eukaryotic expression vector JA4304. When DPV was fused downstream of MAPS (MAPS/DPV) in the DNA vaccine format, a greater amount of DPV protein was produced with a concomitant increase in the DPV-specific immune response, as assayed by a number of methods.

In an effort to understand the biochemical and immunological mechanism(s) underlying the increases in total DPV protein and DPV-specific immune responses following immunization of mice the fusion DPV-AC/MAPS was constructed (SEQ ID NOS: 75 and 76). DPV-AC/MAPS was constructed by fusing in frame the codon-optimized version of DPV (DPV-AC) upstream of MAPS, using the following PCR-based strategy. The DPV-AC gene was PCR amplified using the oligonucleotides DPV-AC-HindIII-sense-I (which adds a restriction site and kozak sequence) and DPV-AC antisense (which removes the DPV stop codon). MAPS was PCR amplified using the oligonucleotides DPV-AC/MAPS fusion (which is a hybrid oligo that anneals to the 3'-end of the DPV-AC sequence and the 5'-end of MAPS) and MAPS-3-BamHI (which adds a restriction site downstream of the MAPS stop codon). The two PCR products, DPV-AC and MAPS with the DPV-AC sequence leader, were then mixed, annealed and subjected to an additional round of PCR using the outside oligonucleotides, DPV-AC-HindIII-sense-I and MAPS-3-BamHI, to generate the DPV-

AC/MAPS fusion. This DNA was directionally cloned into HindIII and BamHI cut JA4304, and purified on a large scale by Qiagen endo-free DNA giga-prep.

The DPV-AC/MAPS fusion DNA has been compared to the DPV, MAPS and MAPS/DPV DNA vaccine constructs following transfection into HEK 293T cells for protein expression levels by Western blot and relative transcript levels by RT-PCR. Western blotting of HEK 293T cell lysates demonstrated that DPV-AC/MAPS plasmid produces an amount of DPV protein similar to that of MAPS/DPV and greater than that produced by the DPV plasmid alone. This result indicates that, when fused either upstream or downstream of DPV, MAPS can increase the level of DPV protein expression. In fact, the levels of DPV-AC/MAPS, MAPS/DPV and MAPS protein are produced at sufficient levels to be visible by coomassie steining. The level of MAPS protein produced by the DPV-AC/MAPS plasmid is approximately equal to that of MAPS plasmid alone and revealed that the amount of DPV produced has been increased to the normal level of MAPS. RT-PCR analysis demonstrated that there was no difference in the amount of DPV-specific transcript in HEK 293T cells transfected with the DPV, MAPS/DPV or DPV-AC/MAPS plasmids. Without being bound by theory, this result suggests that MAPS may be increasing the amount of DPV protein by stabilizing DPV within the cell and preventing its rapid degradation.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1: MTB32A (Ra35 FL)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1872 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GACTACGTTG GTGTAGAAAA ATCCTGCCGC CCGGACCCTT AAGGCTGGGA CAATTTCTGA     60
TAGCTACCCC GACACAGGAG GTTACGGGAT GAGCAATTCG CGCCGCCGCT CACTCAGGTG    120
GTCATGGTTG CTGAGCGTGC TGGCTGCCGT CGGGCTGGGC CTGGCCACGG CGCCGGCCCA    180
GGCGGCCCCG CCGGCCTTGT CGCAGGACCG GTTCGCCGAC TTCCCCGCGC TGCCCCTCGA    240
CCCGTCCGCG ATGGTCGCCC AAGTGGCGCC ACAGGTGGTC AACATCAACA CCAAACTGGG    300
CTACAACAAC GCCGTGGGCG CCGGGACCGG CATCGTCATC GATCCCAACG GTGTCGTGCT    360
GACCAACAAC CACGTGATCG CGGGCGCCAC CGACATCAAT GCGTTCAGCG TCGGCTCCGG    420
CCAAACCTAC GGCGTCGATG TGGTCGGGTA TGACCGCACC CAGGATGTCG CGGTGCTGCA    480
GCTGCGCGGT GCCGGTGGCC TGCCGTCGGC GGCGATCGGT GGCGGCGTCG CGGTTGGTGA    540
GCCCGTCGTC GCGATGGGCA ACAGCGGTGG GCAGGGCGGA ACGCCCCGTG CGGTGCCTGG    600
CAGGGTGGTC GCGCTCGGCC AAACCGTGCA GGCGTCGGAT TCGCTGACCG GTGCCGAAGA    660
GACATTGAAC GGGTTGATCC AGTTCGATGC CGCAATCCAG CCCGGTGATT CGGGCGGGCC    720
CGTCGTCAAC GGCCTAGGAC AGGTGGTCGG TATGAACACG GCCGCGTCCG ATAACTTCCA    780
GCTGTCCCAG GGTGGGCAGG GATTCGCCAT TCCGATCGGG CAGGCGATGG CGATCGCGGG    840
CCAAATCCGA TCGGGTGGGG GGTCACCCAC CGTTCATATC GGGCCTACCG CCTTCCTCGG    900
CTTGGGTGTT GTCGACAACA ACGGCAACGG CGCACGAGTC CAACGCGTGG TCGGAAGCGC    960
TCCGGCGGCA AGTCTCGGCA TCTCCACCGG CGACGTGATC ACCGCGGTCG ACGGCGCTCC   1020
GATCAACTCG GCCACCGCGA TGGCGGACGC GCTTAACGGG CATCATCCCG GTGACGTCAT   1080
CTCGGTGAAC TGGCAAACCA AGTCGGGCGG CACGCGTACA GGGAACGTGA CATTGGCCGA   1140
GGGACCCCCG GCCTGATTTG TCGCGGATAC CACCCGCCGG CCGGCCAATT GGATTGGCGC   1200
CAGCCGTGAT TGCCGCGTGA GCCCCCGAGT TCCGTCTCCC GTGCGCGTGG CATTGTGGAA   1260
GCAATGAACG AGGCAGAACA CAGCGTTGAG CACCCTCCCG TGCAGGGCAG TTACGTCGAA   1320
GGCGGTGTGG TCGAGCATCC GGATGCCAAG GACTTCGGCA GCGCCGCCGC CCTGCCCGCC   1380
GATCCGACCT GGTTTAAGCA CGCCGTCTTC TACGAGGTGC TGGTCCGGGC GTTCTTCGAC   1440
GCCAGCGCGG ACGGTTCCGN CGATCTGCGT GGACTCATCG ATCGCCTCGA CTACCTGCAG   1500
TGGCTTGGCA TCGACTGCAT CTGTTGCCGC CGTTCCTACG ACTCACCGCT GCGCGACGGC   1560
GGTTACGACA TTCGCGACTT CTACAAGGTG CTGCCCGAAT TCGGCACCGT CGACGATTTC   1620
GTCGCCCTGG TCGACACCGC TCACCGGCGA GGTATCCGCA TCATCACCGA CCTGGTGATG   1680
AATCACACCT CGGAGTCGCA CCCCTGGTTT CAGGAGTCCC GCCGCGACCC AGACGGACCG   1740
TACGGTGACT ATTACGTGTG GAGCGACACC AGCGAGCGCT ACACCGACGC CCGGATCATC   1800
TTCGTCGACA CCGAAGAGTC GAACTGGTCA TTCGATCCTG TCCGCCGACA GTTNCTACTG   1860
GCACCGATTC TT                                                       1872
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 355 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2: MTB32A (Ra35FL)

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Met Ser Asn Ser Arg Arg Arg Ser Leu Arg Trp Ser Trp Leu Leu Ser
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Val Leu Ala Ala Val Gly Leu Gly Leu Ala Thr Ala Pro Ala Gln Ala
            20                  25                  30
Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu
        35                  40                  45
Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Ala Pro Gln Val Val
    50                  55                  60
Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr
65                  70                  75                  80
Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val
```

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

<212> DNA
<213> Ra35 mature
<400> SEQ ID NO:3

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aacatcaaca ccaaactggg ctacaacaac gccgtgggcg ccgggaccgg catcgtcatc 180
gatcccaacg gtgtcgtgct gaccaacaac cacgtgatcg cgggcgccac cgacatcaat 240
gcgttcagcg tcggctccgg ccaaacctac ggcgtcgatg tggtcgggta tgaccgcacc 300
caggatgtcg cggtgctgca gctgcgcggt gccggtggcc tgccgtcggc ggcgatcggt 360
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cccggtgagg cgggcgggcc cgtcgtcaac ggcctaggac aggtggtcgg tatgaacacg 600
gccgcgtccg ataacttcca gctgtcccag ggtgggcagg gattcgccat tccgatcggg 660
caggcgatgg cgatcgcggg ccagatccga tcgggtgggg ggtcacccac cgttcatatc 720
gggcctaccg ccttcctcgg cttgggtgtt gtcgacaaca acggcaacgg cgcacgagtc 780
caacgcgtgg tcgggagcgc tccggcggca agtctcggca tctccaccgg cgacgtgatc 840
accgcggtcg acggcgctcc gatcaactcg gccaccgcga tggcggacgc gcttaacggg 900
catcatcccg gtgacgtcat ctcggtgacc tggcaaacca agtcgggcgg cacgcgtaca 960
gggaacgtga cattggccga gggacccccg gcctgagaat tc                   1002
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<212> PRT
<213> Ra35 mature
<400> SEQ ID NO:4

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```
                    20                  25                  30

Gln Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn
        35                  40                  45

Asn Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val
    50                  55                  60

Val Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala
65                  70                  75                  80

Phe Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr
                85                  90                  95

Asp Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly
            100                 105                 110

Leu Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val
        115                 120                 125

Val Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val
    130                 135                 140

Pro Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser
145                 150                 155                 160

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

Ala Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly
            180                 185                 190

Gln Val Val Gly Met Asn Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser
        195                 200                 205

Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile
    210                 215                 220

Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly
225                 230                 235                 240

Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly
                245                 250                 255

Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly
            260                 265                 270

Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn
        275                 280                 285

Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp
    290                 295                 300

Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly
305                 310                 315                 320

Asn Val Thr Leu Ala Glu Gly Pro Pro Ala
                325                 330
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<212> DNA
<213> Ra35FLMutSA
<400> SEQ ID NO:5

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aacatcaaca ccaaactggg ctacaacaac gccgtgggcg ccgggaccgg catcgtcatc 180
gatcccaacg gtgtcgtgct gaccaacaac cacgtgatcg cgggcgccac cgacatcaat 240
```

```
gcgttcagcg tcggctccgg ccaaacctac ggcgtcgatg tggtcgggta tgaccgcacc 300
caggatgtcg cggtgctgca gctgcgcggt gccggtggcc tgccgtcggc ggcgatcggt 360
ggcggcgtcg cggttggtga gcccgtcgtc gcgatgggca acagcggtgg gcagggcgga 420
acgccccgtg cggtgcctgg cagggtggtc gcgctcggcc aaaccgtgca ggcgtcggat 480
tcgctgaccg gtgccgaaga gacattgaac gggttgatcc agttcgatgc cgcgatccag 540
cccggtgatg cgggcgggcc cgtcgtcaac ggcctaggac aggtggtcgg tatgaacacg 600
gccgcgtccg ataacttcca gctgtcccag ggtgggcagg gattcgccat tccgatcggg 660
caggcgatgg cgatcgcggg ccagatccga tcgggtgggg ggtcacccac cgttcatatc 720
gggcctaccg ccttcctcgg cttgggtgtt gtcgacaaca acggcaacgg cgcacgagtc 780
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catcatcccg gtgacgtcat ctcggtgacc tggcaaacca agtcgggcgg cacgcgtaca 960
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```

<212> PRT
<213> Ra35FLMutSA
<400> SEQ ID NO:6

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

Gln Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn
            35                  40                  45

Asn Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val
        50                  55                  60

Val Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala
65                  70                  75                  80

Phe Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr
                85                  90                  95

Asp Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly
            100                 105                 110

Leu Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val
        115                 120                 125

Val Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val
    130                 135                 140

Pro Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser
145                 150                 155                 160

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

Ala Ile Gln Pro Gly Asp Ala Gly Gly Pro Val Val Asn Gly Leu Gly
            180                 185                 190

Gln Val Val Gly Met Asn Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser
        195                 200                 205

Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile
    210                 215                 220

Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly
225                 230                 235                 240

Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly
                245                 250                 255

Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly
```

```
            260                 265                 270

Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn
        275                 280                 285

Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp
    290                 295                 300

Val Ile Ser Val Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly
305                 310                 315                 320

Asn Val Thr Leu Ala Glu Gly Pro Pro Ala
                325                 330
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(2) INFORMATION FOR SEQ ID NO:7: Ra35 (MTB32A N-term)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 615 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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(2) INFORMATION FOR SEQ ID NO:8: Ra35 (MTB32A N-term)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 205 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala Leu

Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Ala Pro Gln Val Val

Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly Thr

Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His Val

Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly Gln

Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val Ala

Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile Gly

Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser Gly

Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala Leu

Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu Thr

Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp Ser
```

Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn Thr

Ala Ala Ser

5

(2) INFORMATION FOR SEQ ID NO:9: Ra12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 447 base pairs
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
15

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CACCGTTCAT ATCGGGCCTA CCGCCTTCCT CGGCTTGGGT GTTGTCGACA ACAACGGCAA   180
CGGCGCACGA GTCCAACGCG TGGTCGGGAG CGCTCCGGCG GCAAGTCTCG GCATCTCCAC   240
CGGCGACGTG ATCACCGCGG TCGACGGCGC TCCGATCAAC TCGGCCACCG CGATGGCGGA   300
CGCGCTTAAC GGGCATCATC CCGGTGACGT CATCTCGGTG AACTGGCAAA CCAAGTCGGG   360
CGGCACGCGT ACAGGGAACG TGACATTGGC CGAGGGACCC CCGGCCTGAT TTCGTCGYGG   420
ATACCACCCG CCGGCCGGCC AATTGGA                                       447
```

25

(2) INFORMATION FOR SEQ ID NO:10: Ra12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 amino acids
30 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
35

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Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln Gly Phe
1               5                   10                  15
Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile Arg Ser
            20                  25                  30
Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe Leu Gly
        35                  40                  45
Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln Arg Val
    50                  55                  60
Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly Asp Val
65                  70                  75                  80
Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala Met Ala
                85                  90                  95
Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val Asn Trp
            100                 105                 110
Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu Ala Glu
        115                 120                 125
Gly Pro Pro Ala
    130
```

55

(2) INFORMATION FOR SEQ ID NO:11: TbH9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 851 base pairs
60 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
65

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CTGCAGGGTG GCGTGGATGA GCGTCACCGC GGGGCAGGCC GAGCTGACCG CCGCCCAGGT    60
CCGGGTTGCT GCGGCGGCCT ACGAGACGGC GTATGGGCTG ACGGTGCCCC CGCCGGTGAT   120
CGCCGAGAAC CGTGCTGAAC TGATGATTCT GATAGCGACC AACCTCTTGG GGCAAAACAC   180
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CCCGGCGATC GCGGTCAACG AGGCCGAATA CGGCGAGATG TGGGCCCAAG ACGCCGCCGC      240
GATGTTTGGC TACGCCGCGG CGACGGCGAC GGCGACGGCG ACGTTGCTGC CGTTCGAGGA      300
GGCGCCGGAG ATGACCAGCG CGGGTGGGCT CCTCGAGCAG GCCGCCGCGG TCGAGGAGGC      360
CTCCGACACC GCCGCGGCGA ACCAGTTGAT GAACAATGTG CCCCAGGCGC TGAAACAGTT      420
GGCCCAGCCC ACGCAGGGCA CCACGCCTTC TTCCAAGCTG GGTGGCCTGT GGAAGACGGT      480
CTCGCCGCAT CGGTCGCCGA TCAGCAACAT GGTGTCGATG GCCAACAACC ACATGTCGAT      540
GACCAACTCG GGTGTGTCGA TGACCAACAC CTTGAGCTCG ATGTTGAAGG GCTTTGCTCC      600
GGCGGCGGCC GCCCAGGCCG TGCAAACCGC GGCGCAAAAC GGGGTCCGGG CGATGAGCTC      660
GCTGGGCAGC TCGCTGGGTT CTTCGGGTCT GGGCGGTGGG GTGGCCGCCA ACTTGGGTCG      720
GGCGGCCTCG GTACGGTATG GTCACCGGGA TGGCGGAAAA TATGCANAGT CTGGTCGGCG      780
GAACGGTGGT CCGGCGTAAG GTTTACCCCC GTTTTCTGGA TGCGGTGAAC TTCGTCAACG      840
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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 263 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12: TbH9

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

(2) INFORMATION FOR SEQ ID NO:13: TBH9FL

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3058 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GATCGTACCC GTGCGAGTGC TCGGGCCGTT TGAGGATGGA GTGCACGTGT CTTTCGTGAT     60
GGCATACCCA GAGATGTTGG CGGCGGCGGC TGACACCCTG CAGAGCATCG GTGCTACCAC    120
TGTGGCTAGC AATGCCGCTG CGGCGGCCCC GACGACTGGG GTGGTGCCCC CCGCTGCCGA    180
TGAGGTGTCG GCGCTGACTG CGGCGCACTT CGCCGCACAT GCGGCGATGT ATCAGTCCGT    240
GAGCGCTCGG GCTGCTGCGA TTCATGACCA GTTCGTGGCC ACCCTTGCCA GCAGCGCCAG    300
CTCGTATGCG GCCACTGAAG TCGCCAATGC GGCGGCGGCC AGCTAAGCCA GGAACAGTCG    360
GCACGAGAAA CCACGAGAAA TAGGGACACG TAATGGTGGA TTTCGGGGCG TTACCACCGG    420
AGATCAACTC CGCGAGGATG TACGCCGGCC CGGGTTCGGC CTCGCTGGTG GCCGCGGCTC    480
AGATGTGGGA CAGCGTGGCG AGTGACCTGT TTTCGGCCGC GTCGGCGTTT CAGTCGGTGG    540
TCTGGGGTCT GACGGTGGGG TCGTGGATAG GTTCGTCGGC GGGTCTGATG GTGGCGGCGG    600
CCTCGCCGTA TGTGGCGTGG ATGAGCGTCA CCGCGGGGCA GGCCGAGCTG ACCGCCGCCC    660
AGGTCCGGGT TGCTGCGGCG GCCTACGAGA CGGCGTATGG GCTGACGGTG CCCCCGCCGG    720
TGATCGCCGA GAACCGTGCT GAACTGATGA TTCTGATAGC GACCAACCTC TTGGGGCAAA    780
ACACCCCGGC GATCGCGGTC AACGAGGCCG AATACGGCGA GATGTGGGCC CAAGACGCCG    840
CCGCGATGTT TGGCTACGCC GCGGCGACGG CGACGGCGAC GGCGACGTTG CTGCCGTTCG    900
AGGAGGCGCC GGAGATGACC AGCGCGGGTG GGCTCCTCGA GCAGGCCGCC GCGGTCGAGG    960
AGGCCTCCGA CACCGCCGCG GCGAACCAGT TGATGAACAA TGTGCCCCAG GCGCTGCAAC   1020
AGCTGGCCCA GCCCACGCAG GGCACCACGC CTTCTTCCAA GCTGGGTGGC CTGTGGAAGA   1080
CGGTCTCGCC GCATCGGTCG CCGATCAGCA ACATGGTGTC GATGGCCAAC AACCACATGT   1140
CGATGACCAA CTCGGGTGTG TCGATGACCA ACACCTTGAG CTCGATGTTG AAGGGCTTTG   1200
CTCCGGCGGC GGCCGCCCAG GCCGTGCAAA CCGCGGCGCA AAACGGGGTC CGGGCGATGA   1260
GCTCGCTGGG CAGCTCGCTG GGTTCTTCGG GTCTGGGCGG TGGGGTGGCC GCCAACTTGG   1320
GTCGGGCGGC CTCGGTCGGT TCGTTGTCGG TGCCGCAGGC CTGGGCCGCG GCCAACCAGG   1380
CAGTCACCCC GGCGGCGCGG GCGCTGCCGC TGACCAGCCT GACCAGCGCC GCGGAAAGAG   1440
GGCCCGGGCA GATGCTGGGC GGGCTGCCGG TGGGGCAGAT GGGCGCCAGG GCCGGTGGTG   1500
GGCTCAGTGG TGTGCTGCGT GTTCCGCCGC GACCCTATGT GATGCCGCAT TCTCCGGCGG   1560
CCGGCTAGGA GAGGGGGCGC AGACTGTCGT TATTTGACCA GTGATCGGCG GTCTCGGTGT   1620
TTCCGCGGCC GGCTATGACA ACAGTCAATG TGCATGACAA GTTACAGGTA TTAGGTCCAG   1680
GTTCAACAAG GAGACAGGCA ACATGGCCTC ACGTTTTATG ACGGATCCGC ACGCGATGCG   1740
GGACATGGCG GGCCGTTTTG AGGTGCACGC CCAGACGGTG GAGGACGAGG CTCGCCGGAT   1800
GTGGGCGTCC GCGCAAAACA TTTCCGGTGC GGGCTGGAGT GGCATGGCCG AGGCGACCTC   1860
GCTAGACACC ATGGCCCAGA TGAATCAGGC GTTTCGCAAC ATCGTGAACA TGCTGCACGG   1920
GGTGCGTGAC GGGCTGGTTC GCGACGCCAA CAACTACGAG CAGCAAGAGC AGGCCTCCCA   1980
```

```
GCAGATCCTC AGCAGCTAAC GTCAGCCGCT GCAGCACAAT ACTTTTACAA GCGAAGGAGA    2040
ACAGGTTCGA TGACCATCAA CTATCAATTC GGGGATGTCG ACGCTCACGG CGCCATGATC    2100
CGCGCTCAGG CCGGGTTGCT GGAGGCCGAG CATCAGGCCA TCATTCGTGA TGTGTTGACC    2160
GCGAGTGACT TTTGGGGCGG CGCCGGTTCG GCGGCCTGCC AGGGGTTCAT TACCCAGTTG    2220
GGCCGTAACT TCCAGGTGAT CTACGAGCAG GCCAACGCCC ACGGGCAGAA GGTGCAGGCT    2280
GCCGGCAACA ACATGGCGCA AACCGACAGC GCCGTCGGCT CCAGCTGGGC CTGACACCAG    2340
GCCAAGGCCA GGGACGTGGT GTACGAGTGA AGTTCCTCGC GTGATCCTTC GGGTGGCAGT    2400
CTAAGTGGTC AGTGCTGGGG TGTTGGTGGT TTGCTGCTTG GCGGGTTCTT CGGTGCTGGT    2460
CAGTGCTGCT CGGGCTCGGG TGAGGACCTC GAGGCCCAGG TAGCGCCGTC CTTCGATCCA    2520
TTCGTCGTGT TGTTCGGCGA GGACGGCTCC GACGAGGCGG ATGATCGAGG CGCGGTCGGG    2580
GAAGATGCCC ACGACGTCGG TTCGGCGTCG TACCTCTCGG TTGAGGCGTT CCTGGGGGTT    2640
GTTGGACCAG ATTTGGCGCC AGATCTGCTT GGGGAAGGCG GTGAACGCCA GCAGGTCGGT    2700
GCGGGCGGTG TCGAGGTGCT CGGCCACCGC GGGGAGTTTG TCGGTCAGAG CGTCGAGTAC    2760
CCGATCATAT TGGGCAACAA CTGATTCGGC GTCGGGCTGG TCGTAGATGG AGTGCAGCAG    2820
GGTGCGCACC CACGGCCAGG AGGGCTTCGG GGTGGCTGCC ATCAGATTGG CTGCGTAGTG    2880
GGTTCTGCAG CGCTGCCAGG CCGCTGCGGG CAGGGTGGCG CCGATCGCGG CCACCAGGCC    2940
GGCGTGGGCG TCGCTGGTGA CCAGCGCGAC CCCGGACAGG CCGCGGGCGA CCAGGTCGCG    3000
GAAGAACGCC AGCCAGCCGG CCCCGTCCTC GGCGGAGGTG ACCTGGATGC CCAGGATC      3058
```

(2) INFORMATION FOR SEQ ID NO:14: TbH9FL

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 391 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

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Met Val Asp Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met
1               5                   10                  15

Tyr Ala Gly Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp
            20                  25                  30

Asp Ser Val Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser
        35                  40                  45

Val Val Trp Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly
    50                  55                  60

Leu Met Val Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr
65                  70                  75                  80

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

Ala Tyr Glu Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala
            100                 105                 110
```

Glu Asn Arg Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly
115 120 125

Gln Asn Thr Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met
130 135 140

Trp Ala Gln Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala
145 150 155 160

Thr Ala Thr Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr
165 170 175

Ser Ala Gly Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser
180 185 190

Asp Thr Ala Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu
195 200 205

Gln Gln Leu Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu
210 215 220

Gly Gly Leu Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn
225 230 235 240

Met Val Ser Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val
245 250 255

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

Ala Ala Ala Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala
275 280 285

Met Ser Ser Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly
290 295 300

Val Ala Ala Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val
305 310 315 320

Pro Gln Ala Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg
325 330 335

Ala Leu Pro Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly
340 345 350

Gln Met Leu Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly
355 360 365

Gly Gly Leu Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met
370 375 380

Pro His Ser Pro Ala Ala Gly
385 390

<210> SEQ ID NO:15
<211> 2287
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion
protein Mtb72F(Ra12-TbH9-Ra35 or Mtb32-Mtb39
fusion)

tctagaaata attttgttta ctttaagaan ganatataca t atg cat cac cat cac 56
Met His His His His
1 5

cat cac acg gcc gcg tcc gat aac ttc cag ctg tcc cag ggt ggg cag 104

```
His His Thr Ala Ala Ser Asp Asn Phe Gln Leu Ser Gln Gly Gly Gln
                10                  15                  20

gga ttc gcc att ccg atc ggg cag gcg atg gcg atc gcg ggc cag atc      152
Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala Ile Ala Gly Gln Ile
            25                  30                  35

cga tcg ggt ggg ggg tca ccc acc gtt cat atc ggg cct acc gcc ttc      200
Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile Gly Pro Thr Ala Phe
        40                  45                  50

ctc ggc ttg ggt gtt gtc gac aac aac ggc aac ggc gca cga gtc caa      248
Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn Gly Ala Arg Val Gln
    55                  60                  65

cgc gtg gtc ggg agc gct ccg gcg gca agt ctc ggc atc tcc acc ggc      296
Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu Gly Ile Ser Thr Gly
 70                  75                  80                  85

gac gtg atc acc gcg gtc gac ggc gct ccg atc aac tcg gcc acc gcg      344
Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile Asn Ser Ala Thr Ala
                90                  95                 100

atg gcg gac gcg ctt aac ggg cat cat ccc ggt gac gtc atc tcg gtg      392
Met Ala Asp Ala Leu Asn Gly His His Pro Gly Asp Val Ile Ser Val
            105                 110                 115

acc tgg caa acc aag tcg ggc ggc acg cgt aca ggg aac gtg aca ttg      440
Thr Trp Gln Thr Lys Ser Gly Gly Thr Arg Thr Gly Asn Val Thr Leu
        120                 125                 130

gcc gag gga ccc ccg gcc gaa ttc atg gtg gat ttc ggg gcg tta cca      488
Ala Glu Gly Pro Pro Ala Glu Phe Met Val Asp Phe Gly Ala Leu Pro
    135                 140                 145

ccg gag atc aac tcc gcg agg atg tac gcc ggc ccg ggt tcg gcc tcg      536
Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser
150                 155                 160                 165

ctg gtg gcc gcg gct cag atg tgg gac agc gtg gcg agt gac ctg ttt      584
Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe
                170                 175                 180

tcg gcc gcg tcg gcg ttt cag tcg gtg gtc tgg ggt ctg acg gtg ggg      632
Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly
            185                 190                 195

tcg tgg ata ggt tcg tcg gcg ggt ctg atg gtg gcg gcg gcc tcg ccg      680
Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro
        200                 205                 210

tat gtg gcg tgg atg agc gtc acc gcg ggg cag gcc gag ctg acc gcc      728
Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala
    215                 220                 225

gcc cag gtc cgg gtt gct gcg gcg gcc tac gag acg gcg tat ggg ctg      776
Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu
230                 235                 240                 245

acg gtg ccc ccg ccg gtg atc gcc gag aac cgt gct gaa ctg atg att      824
Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile
                250                 255                 260

ctg ata gcg acc aac ctc ttg ggg caa aac acc ccg gcg atc gcg gtc      872
Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val
            265                 270                 275

aac gag gcc gaa tac ggc gag atg tgg gcc caa gac gcc gcc gcg atg      920
```

```
Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met
        280                 285                 290

ttt ggc tac gcc gcg gcg acg gcg acg gcg acg gcg acg ttg ctg ccg      968
Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro
    295                 300                 305

ttc gag gag gcg ccg gag atg acc agc gcg ggt ggg ctc ctc gag cag     1016
Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln
310                 315                 320                 325

gcc gcc gcg gtc gag gag gcc tcc gac acc gcc gcg gcg aac cag ttg     1064
Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu
                330                 335                 340

atg aac aat gtg ccc cag gcg ctg caa cag ctg gcc cag ccc acg cag     1112
Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr Gln
            345                 350                 355

ggc acc acg cct tct tcc aag ctg ggt ggc ctg tgg aag acg gtc tcg     1160
Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser
        360                 365                 370

ccg cat cgg tcg ccg atc agc aac atg gtg tcg atg gcc aac aac cac     1208
Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His
    375                 380                 385

atg tcg atg acc aac tcg ggt gtg tcg atg acc aac acc ttg agc tcg     1256
Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser
390                 395                 400                 405

atg ttg aag ggc ttt gct ccg gcg gcg gcc cgc cag gcc gtg caa acc     1304
Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Arg Gln Ala Val Gln Thr
                410                 415                 420

gcg gcg caa aac ggg gtc cgg gcg atg agc tcg ctg ggc agc tcg ctg     1352
Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu
            425                 430                 435

ggt tct tcg ggt ctg ggc ggt ggg gtg gcc gcc aac ttg ggt cgg gcg     1400
Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala
        440                 445                 450

gcc tcg gtc ggt tcg ttg tcg gtg ccg cag gcc tgg gcc gcg gcc aac     1448
Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn
    455                 460                 465

cag gca gtc acc ccg gcg gcg cgg gcg ctg ccg ctg acc agc ctg acc     1496
Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr
470                 475                 480                 485

agc gcc gcg gaa aga ggg ccc ggg cag atg ctg ggc ggg ctg ccg gtg     1544
Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro Val
                490                 495                 500

ggg cag atg ggc gcc agg gcc ggt ggt ggg ctc agt ggt gtg ctg cgt     1592
Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu Arg
            505                 510                 515

gtt ccg ccg cga ccc tat gtg atg ccg cat tct ccg gca gcc ggc gat     1640
Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly Asp
        520                 525                 530

atc gcc ccg ccg gcc ttg tcg cag gac cgg ttc gcc gac ttc ccc gcg     1688
Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala
    535                 540                 545

ctg ccc ctc gac ccg tcc gcg atg gtc gcc caa gtg ggg cca cag gtg     1736
```

```
Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val
550                 555                 560                 565

gtc aac atc aac acc aaa ctg ggc tac aac aac gcc gtg ggc gcc ggg     1784
Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly
                570                 575                 580

acc ggc atc gtc atc gat ccc aac ggt gtc gtg ctg acc aac aac cac     1832
Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His
            585                 590                 595

gtg atc gcg ggc gcc acc gac atc aat gcg ttc agc gtc ggc tcc ggc     1880
Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly
        600                 605                 610

caa acc tac ggc gtc gat gtg gtc ggg tat gac cgc acc cag gat gtc     1928
Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val
    615                 620                 625

gcg gtg ctg cag ctg cgc ggt gcc ggt ggc ctg ccg tcg gcg gcg atc     1976
Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile
630                 635                 640                 645

ggt ggc ggc gtc gcg gtt ggt gag ccc gtc gtc gcg atg ggc aac agc     2024
Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser
                650                 655                 660

ggt ggg cag ggc gga acg ccc cgt gcg gtg cct ggc agg gtg gtc gcg     2072
Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala
            665                 670                 675

ctc ggc caa acc gtg cag gcg tcg gat tcg ctg acc ggt gcc gaa gag     2120
Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu
        680                 685                 690

aca ttg aac ggg ttg atc cag ttc gat gcc gcg atc cag ccc ggt gat     2168
Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp
    695                 700                 705

tcg ggc ggg ccc gtc gtc aac ggc cta gga cag gtg gtc ggt atg aac     2216
Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn
710                 715                 720                 725

acg gcc gcg tcc taggatatcc atcacactgg cggccgctcg agcagatccg         2268
Thr Ala Ala Ser

gntgtaacaa agcccgaaa                                                2287


<210> SEQ ID NO:16
<211> 729
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion
      protein Mtb72F (Ra12-TbH9-Ra35 or Mtb32-Mtb39
      fusion)

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

Ser Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
             20                  25                  30

Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
         35                  40                  45

Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
     50                  55                  60
```

Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
65 70 75 80

Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
85 90 95

Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
100 105 110

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

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

Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly
145 150 155 160

Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
165 170 175

Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
180 185 190

Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
195 200 205

Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
210 215 220

Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
225 230 235 240

Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg
245 250 255

Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
260 265 270

Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
275 280 285

Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr
290 295 300

Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
305 310 315 320

Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
325 330 335

Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
340 345 350

Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
355 360 365

Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser
370 375 380

Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
385 390 395 400

Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Arg
405 410 415

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser
420 425 430

```
Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala
          435                 440                 445

Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala
     450                 455                 460

Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro
465                 470                 475                 480

Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu
                485                 490                 495

Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu
            500                 505                 510

Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser
        515                 520                 525

Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe
    530                 535                 540

Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln
545                 550                 555                 560

Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn
                565                 570                 575

Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val
            580                 585                 590

Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe
        595                 600                 605

Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp
    610                 615                 620

Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu
625                 630                 635                 640

Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val
                645                 650                 655

Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro
            660                 665                 670

Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu
        675                 680                 685

Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala
    690                 695                 700

Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln
705                 710                 715                 720

Val Val Gly Met Asn Thr Ala Ala Ser
                725
```

<210> SEQ ID NO:17
<211> 2190
<212> DNA
<213> Mtb72FMutSA

```
atgcatcacc atcaccatca cacggccgcg tccgataact tccagctgtc ccagggtggg 60
cagggattcg ccattccgat cgggcaggcg atggcgatcg cgggccagat ccgatcgggt 120
ggggggtcac ccaccgttca tatcgggcct accgccttcc tcggcttggg tgttgtcgac 180
aacaacggca acggcgcacg agtccaacgc gtggtcggga gcgctccggc ggcaagtctc 240
```

ggcatctcca ccggcgacgt gatcaccgcg gtcgacggcg ctccgatcaa ctcggccacc 300
gcgatggcgg acgcgcttaa cgggcatcat cccggtgacg tcatctcggt gacctggcaa 360
accaagtcgg gcggcacgcg tacagggaac gtgacattgg ccgagggacc cccggccgaa 420
ttcatggtgg atttcggggc gttaccaccg gagatcaact ccgcgaggat gtacgccggc 480
ccgggttcgg cctcgctggt ggccgcggct cagatgtggg acagcgtggc gagtgacctg 540
ttttcggccg cgtcggcgtt tcagtcggtg gtctggggtc tgacggtggg gtcgtggata 600
ggttcgtcgg cgggtctgat ggtggcggcg gcctcgccgt atgtggcgtg gatgagcgtc 660
accgcggggc aggccgagct gaccgccgcc caggtccggg ttgctgcggc ggcctacgag 720
acggcgtatg ggctgacggt gcccccgccg gtgatcgccg agaaccgtgc tgaactgatg 780
attctgatag cgaccaacct cttggggcaa aacacccccgg cgatcgcggt caacgaggcc 840
gaatacggcg agatgtgggc ccaagacgcc gccgcgatgt ttggctacgc cgcggcgacg 900
gcgacggcga cggcgacgtt gctgccgttc gaggaggcgc cggagatgac cagcgcgggt 960
gggctcctcg agcaggccgc cgcggtcgag gaggcctccg acaccgccgc ggcgaaccag 1020
ttgatgaaca atgtgcccca ggcgctgcaa cagctggccc agcccacgca gggcaccacg 1080
ccttcttcca agctgggtgg cctgtggaag acggtctcgc cgcatcggtc gccgatcagc 1140
aacatggtgt cgatggccaa caaccacatg tcgatgacca actcgggtgt gtcgatgacc 1200
aacaccttga gctcgatgtt gaagggcttt gctccggcgg cggccgccca ggccgtgcaa 1260
accgcggcgc aaaacggggt ccgggcgatg agctcgctgg gcagctcgct gggttcttcg 1320
ggtctgggcg gtggggtggc cgccaacttg ggtcgggcgg cctcggtcgg ttcgttgtcg 1380
gtgccgcagg cctgggccgc ggccaaccag gcagtcaccc cggcggcgcg ggcgctgccg 1440
ctgaccagcc tgaccagcgc cgcggaaaga gggcccgggc agatgctggg cgggctgccg 1500
gtggggcaga tgggcgccag ggccggtggt gggctcagtg gtgtgctgcg tgttccgccg 1560
cgaccctatg tgatgccgca ttctccggca gccggcgata tcgccccgcc ggccttgtcg 1620
caggaccggt tcgccgactt ccccgcgctg cccctcgacc cgtccgcgat ggtcgcccaa 1680
gtggggccac aggtggtcaa catcaacacc aaactgggct acaacaacgc cgtgggcgcc 1740
gggaccggca tcgtcatcga tcccaacggt gtcgtgctga ccaacaacca cgtgatcgcg 1800
ggcgccaccg acatcaatgc gttcagcgtc ggctccggcc aaacctacgg cgtcgatgtg 1860
gtcgggtatg accgcaccca ggatgtcgcg gtgctgcagc tgcgcggtgc cggtggcctg 1920
ccgtcggcgg cgatcggtgg cggcgtcgcg gttggtgagc ccgtcgtcgc gatgggcaac 1980
agcggtgggc agggcggaac gccccgtgcg gtgcctggca gggtggtcgc gctcggccaa 2040
accgtgcagg cgtcggattc gctgaccggt gccgaagaga cattgaacgg gttgatccag 2100
ttcgatgccg cgatccagcc cggtgatgcg ggcgggcccg tcgtcaacgg cctaggacag 2160
gtggtcggta tgaacacggc cgcgtcctag 2190

<210> SEQ ID NO:18
<211> 729
<212> PRT
<213> Mtb72FMutSA

Met His His His His His His Thr Ala Ala Ser Asp Asn Phe Gln Leu
5 10 15

Ser Gln Gly Gly Gln Gly Phe Ala Ile Pro Ile Gly Gln Ala Met Ala
20 25 30

Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
35 40 45

Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
50 55 60

Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
65 70 75 80

Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
85 90 95

Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
100 105 110

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

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

Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly

145 150 155 160

Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
165 170 175

5

Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
180 185 190

Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
10 195 200 205

Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
210 215 220

15 Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
225 230 235 240

Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg
245 250 255

20

Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
260 265 270

Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
25 275 280 285

Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr
290 295 300

30 Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
305 310 315 320

Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
325 330 335

35

Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
340 345 350

Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
40 355 360 365

Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser
370 375 380

45 Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
385 390 395 400

Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala
405 410 415

50

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser
420 425 430

Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala
55 435 440 445

Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala
450 455 460

60 Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro
465 470 475 480

Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu
485 490 495

65

Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu
500 505 510

Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser
515 520 525

Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe
530 535 540

Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln
545 550 555 560

Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn
565 570 575

Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val
580 585 590

Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe
595 600 605

Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp
610 615 620

Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu
625 630 635 640

Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val
645 650 655

Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro
660 665 670

Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu
675 680 685

Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala
690 695 700

Ile Gln Pro Gly Asp Ala Gly Gly Pro Val Val Asn Gly Leu Gly Gln
705 710 715 720

Val Val Gly Met Asn Thr Ala Ala Ser
725

<210> SEQ ID NO:19
<211> 1797
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:bi-fusion
protein TbH9-Ra35 (designated Mtb59f)
<222> (1)..(1791)

cat atg cat cac cat cac cat cac atg gtg gat ttc ggg gcg tta cca 48
His Met His His His His His His Met Val Asp Phe Gly Ala Leu Pro
1 5 10 15

ccg gag atc aac tcc gcg agg atg tac gcc ggc ccg ggt tcg gcc tcg 96
Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser
20 25 30

ctg gtg gcc gcg gct cag atg tgg gac agc gtg gcg agt gac ctg ttt 144
Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe
35 40 45

tcg gcc gcg tcg gcg ttt cag tcg gtg gtc tgg ggt ctg acg gtg ggg 192
Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly
50 55 60

tcg tgg ata ggt tcg tcg gcg ggt ctg atg gtg gcg gcg gcc tcg ccg 240

```
Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro
 65                  70                  75                  80

tat gtg gcg tgg atg agc gtc acc gcg ggg cag gcc gag ctg acc gcc     288
Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala
                 85                  90                  95

gcc cag gtc cgg gtt gct gcg gcg gcc tac gag acg gcg tat ggg ctg     336
Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu
            100                 105                 110

acg gtg ccc ccg ccg gtg atc gcc gag aac cgt gct gaa ctg atg att     384
Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile
        115                 120                 125

ctg ata gcg acc aac ctc ttg ggg caa aac acc ccg gcg atc gcg gtc     432
Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val
    130                 135                 140

aac gag gcc gaa tac ggc gag atg tgg gcc caa gac gcc gcc gcg atg     480
Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met
145                 150                 155                 160

ttt ggc tac gcc gcg gcg acg gcg acg gcg acg gcg acg ttg ctg ccg     528
Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro
                165                 170                 175

ttc gag gag gcg ccg gag atg acc agc gcg ggt ggg ctc ctc gag cag     576
Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln
            180                 185                 190

gcc gcc gcg gtc gag gag gcc tcc gac acc gcc gcg gcg aac cag ttg     624
Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu
        195                 200                 205

atg aac aat gtg ccc cag gcg ctg caa cag ctg gcc cag ccc acg cag     672
Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr Gln
    210                 215                 220

ggc acc acg cct tct tcc aag ctg ggt ggc ctg tgg aag acg gtc tcg     720
Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser
225                 230                 235                 240

ccg cat cgg tcg ccg atc agc aac atg gtg tcg atg gcc aac aac cac     768
Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His
                245                 250                 255

atg tcg atg acc aac tcg ggt gtg tcg atg acc aac acc ttg agc tcg     816
Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser
            260                 265                 270

atg ttg aag ggc ttt gct ccg gcg gcg gcc gcc cag gcc gtg caa acc     864
Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala Gln Ala Val Gln Thr
        275                 280                 285

gcg gcg caa aac ggg gtc cgg gcg atg agc tcg ctg ggc agc tcg ctg     912
Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu
    290                 295                 300

ggt tct tcg ggt ctg ggc ggt ggg gtg gcc gcc aac ttg ggt cgg gcg     960
Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala
305                 310                 315                 320

gcc tcg gtc ggt tcg ttg tcg gtg ccg cag gcc tgg gcc gcg gcc aac    1008
Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn
                325                 330                 335

cag gca gtc acc ccg gcg gcg cgg gcg ctg ccg ctg acc agc ctg acc    1056
```

```
Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr
            340                 345                 350

agc gcc gcg gaa aga ggg ccc ggg cag atg ctg ggc ggg ctg ccg gtg    1104
Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro Val
        355                 360                 365

ggg cag atg ggc gcc agg gcc ggt ggt ggg ctc agt ggt gtg ctg cgt    1152
Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu Arg
    370                 375                 380

gtt ccg ccg cga ccc tat gtg atg ccg cat tct ccg gca gcc ggc gat    1200
Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly Asp
385                 390                 395                 400

atc gcc ccg ccg gcc ttg tcg cag gac cgg ttc gcc gac ttc ccc gcg    1248
Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala
                405                 410                 415

ctg ccc ctc gac ccg tcc gcg atg gtc gcc caa gtg ggg cca cag gtg    1296
Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val
            420                 425                 430

gtc aac atc aac acc aaa ctg ggc tac aac aac gcc gtg ggc gcc ggg    1344
Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly
        435                 440                 445

acc ggc atc gtc atc gat ccc aac ggt gtc gtg ctg acc aac aac cac    1392
Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His
    450                 455                 460

gtg atc gcg ggc gcc acc gac atc aat gcg ttc agc gtc ggc tcc ggc    1440
Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly
465                 470                 475                 480

caa acc tac ggc gtc gat gtg gtc ggg tat gac cgc acc cag gat gtc    1488
Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val
                485                 490                 495

gcg gtg ctg cag ctg cgc ggt gcc ggt ggc ctg ccg tcg gcg gcg atc    1536
Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile
            500                 505                 510

ggt ggc ggc gtc gcg gtt ggt gag ccc gtc gtc gcg atg ggc aac agc    1584
Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser
        515                 520                 525

ggt ggg cag ggc gga acg ccc cgt gcg gtg cct ggc agg gtg gtc gcg    1632
Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala
    530                 535                 540

ctc ggc caa acc gtg cag gcg tcg gat tcg ctg acc ggt gcc gaa gag    1680
Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu
545                 550                 555                 560

aca ttg aac ggg ttg atc cag ttc gat gcc gcg atc cag ccc ggt gat    1728
Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp
                565                 570                 575

tcg ggc ggg ccc gtc gtc aac ggc cta gga cag gtg gtc ggt atg aac    1776
Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn
            580                 585                 590

acg gcc gcg tcc taggatatc                                          1797
Thr Ala Ala Ser
        595
```

```
<210> SEQ ID NO:20
<211> 596
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:bi-fusion
      protein TbH9-Ra35 (designated Mtb59f)

His Met His His His His His His Met Val Asp Phe Gly Ala Leu Pro
 1               5                  10                  15

Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly Pro Gly Ser Ala Ser
            20                  25                  30

Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val Ala Ser Asp Leu Phe
        35                  40                  45

Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp Gly Leu Thr Val Gly
    50                  55                  60

Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val Ala Ala Ala Ser Pro
65                  70                  75                      80

Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln Ala Glu Leu Thr Ala
                85                  90                  95

Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu Thr Ala Tyr Gly Leu
            100                 105                 110

Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg Ala Glu Leu Met Ile
        115                 120                 125

Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr Pro Ala Ile Ala Val
    130                 135                 140

Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln Asp Ala Ala Ala Met
145                 150                 155                     160

Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr Ala Thr Leu Leu Pro
                165                 170                 175

Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly Gly Leu Leu Glu Gln
            180                 185                 190

Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala Ala Ala Asn Gln Leu
        195                 200                 205

Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu Ala Gln Pro Thr Gln
    210                 215                 220

Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu Trp Lys Thr Val Ser
225                 230                 235                     240
Pro His Arg Ser Pro Ile Ser Asn Met Val Ser Met Ala Asn Asn His
                245                 250                 255

Met Ser Met Thr Asn Ser Gly Val Ser Met Thr Asn Thr Leu Ser Ser
            260                 265                 270

Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala Gln Ala Val Gln Thr
        275                 280                 285

Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser Leu Gly Ser Ser Leu
    290                 295                 300

Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala Asn Leu Gly Arg Ala
305                 310                 315                     320

Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala Trp Ala Ala Ala Asn
                325                 330                 335
```

```
Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro Leu Thr Ser Leu Thr
            340                 345                 350

Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu Gly Gly Leu Pro Val
        355                 360                 365

Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu Ser Gly Val Leu Arg
    370                 375                 380

Val Pro Pro Arg Pro Tyr Val Met Pro His Ser Pro Ala Ala Gly Asp
385                 390                 395                 400

Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe Ala Asp Phe Pro Ala
                405                 410                 415

Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln Val Gly Pro Gln Val
            420                 425                 430

Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn Ala Val Gly Ala Gly
        435                 440                 445

Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val Leu Thr Asn Asn His
    450                 455                 460

Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe Ser Val Gly Ser Gly
465                 470                 475                 480

Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp Arg Thr Gln Asp Val
                485                 490                 495

Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu Pro Ser Ala Ala Ile
            500                 505                 510

Gly Gly Gly Val Ala Val Gly Glu Pro Val Val Ala Met Gly Asn Ser
        515                 520                 525

Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro Gly Arg Val Val Ala
    530                 535                 540

Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu Thr Gly Ala Glu Glu
545                 550                 555                 560

Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala Ile Gln Pro Gly Asp
                565                 570                 575

Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln Val Val Gly Met Asn
            580                 585                 590

Thr Ala Ala Ser
        595
```

(2) INFORMATION FOR SEQ ID NO:21: DPV (MTB8.4)

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 500 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
CGTGGCAATG TCGTTGACCG TCGGGGCCGG GGTCGCCTCC GCAGATCCCG TGGACGCGGT     60
CATTAACACC ACCTGCAATT ACGGGCAGGT AGTAGCTGCG CTCAACGCGA CGGATCCGGG    120
GGCTGCCGCA CAGTTCAACG CCTCACCGGT GGCGCAGTCC TATTTGCGCA ATTTCCTCGC    180
CGCACCGCCA CCTCAGCGCG CTGCCATGGC CGCGCAATTG CAAGCTGTGC CGGGGGCGGC    240
ACAGTACATC GGCCTTGTCG AGTCGGTTGC CGGCTCCTGC AACAACTATT AAGCCCATGC    300
```

```
GGGCCCCATC CCGCGACCCG GCATCGTCGC CGGGGCTAGG CCAGATTGCC CCGCTCCTCA    360
ACGGGCCGCA TCCCGCGACC CGGCATCGTC GCCGGGGCTA GGCCAGATTG CCCCGCTCCT    420
CAACGGGCCG CATCTCGTGC CGAATTCCTG CAGCCCGGGG GATCCACTAG TTCTAGAGCG    480
GCCGCCACCG CGGTGGAGCT                                                500
```

(2) INFORMATION FOR SEQ ID NO:22: DPV (MTB8.4)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Val Ala Met Ser Leu Thr Val Gly Ala Gly Val Ala Ser Ala Asp Pro
1               5                   10                  15
Val Asp Ala Val Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val Val Ala
            20                  25                  30
Ala Leu Asn Ala Thr Asp Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser
        35                  40                  45
Pro Val Ala Gln Ser Tyr Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro
    50                  55                  60
Gln Arg Ala Ala Met Ala Ala Gln Leu Gln Ala Val Pro Gly Ala Ala
65                  70                  75                  80
Gln Tyr Ile Gly Leu Val Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:23: MSL (MTB9.8)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 585 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Mycobacterium* tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
TGGATTCCGA TAGCGGTTTC GGCCCCTCGA CGGGCGACCA CGGCGCGCAG GCCTCCGAAC     60
GGGGGGCCGG GACGCTGGGA TTCGCCGGGA CCGCAACCAA AGAACGCCGG GTCCGGGCGG    120
TCGGGCTGAC CGCACTGGCC GGTGATGAGT TCGGCAACGG CCCCCGGATG CCGATGGTGC    180
CGGGGACCTG GGAGCAGGGC AGCAACGAGC CCGAGGCGCC CGACGGATCG GGGAGAGGGG    240
GAGGCGACGG CTTACCGCAC GACAGCAAGT AACCGAATTC CGAATCACGT GGACCCGTAC    300
GGGTCGAAAG GAGAGATGTT ATGAGCCTTT TGGATGCTCA TATCCCACAG TTGGTGGCCT    360
CCCAGTCGGC GTTTGCCGCC AAGGCGGGGC TGATGCGGCA CACGATCGGT CAGGCCGAGC    420
AGGCGGCGAT GTCGGCTCAG GCGTTTCACC AGGGGGAGTC GTCGGCGGCG TTTCAGGCCG    480
CCCATGCCCG GTTTGTGGCG GCGGCCGCCA AAGTCAACAC CTTGTTGGAT GTCGCGCAGG    540
CGAATCTGGG TGAGGCCGCC GGTACCTATG TGGCCGCCGA TGCTG                    585
```

(2) INFORMATION FOR SEQ ID NO:24: MSL (MTB9.8)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Met Ser Leu Leu Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser
 1               5                  10                  15
Ala Phe Ala Ala Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala
            20                  25                  30
Glu Gln Ala Ala Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser
        35                  40                  45
Ala Ala Phe Gln Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys
    50                  55                  60
Val Asn Thr Leu Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala
65                  70                  75                  80
Gly Thr Tyr Val Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly
                85                  90                  95
Phe
```

(2) INFORMATION FOR SEQ ID NO:25: MTI (MTB9.9A)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1742 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Mycobacterium* tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
CCGCTCTCTT TCAACGTCAT AAGTTCGGTG GGCCAGTCGG CCGCGCGTGC ATATGGCACC     60
AATAACGCGT GTCCCATGGA TACCCGGACC GCACGACGGT AGAGCGGATC AGCGCAGCCG    120
GTGCCGAACA CTACCGCGTC CACGCTCAGC CCTGCCGCGT TGCGGAAGAT CGAGCCCAGG    180
TTCTCATGGT CGTTAACGCC TTCCAACACT GCGACGGTGC GCGCCCCGGC GACCACCTGA    240
GCAACGCTCG GCTCCGGCAC CCGGCGCGCG GCTGCCAACA CCCCACGATT GAGATGGAAG    300
CCGATCACCC GTGCCATGAC ATCAGCCGAC GCTCGATAGT ACGGCGCGCC GACACCGGCC    360
AGATCATCCT TGAGCTCGGC CAGCCGGCGG TCGGTGCCGA ACAGCGCCAG CGGCGTGAAC    420
CGTGAGGCCA GCATGCGCTG CACCACCAGC ACACCCTCGG CGATCACCAA CGCCTTGCCG    480
GTCGGCAGAT CGGGACNACN GTCGATGCTG TTCAGGTCAC GGAAATCGTC GAGCCGTGGG    540
TCGTCGGGAT CGCAGACGTC CTGAACATCG AGGCCGTCGG GGTGCTGGGC ACAACGGCCT    600
TCGGTCACGG GCTTTCGTCG ACCAGAGCCA GCATCAGATC GGCGGCGCTG CGCAGGATGT    660
CACGCTCGCT GCGGTTCAGC GTCGCGAGCC GCTCAGCCAG CCACTCTTGC AGAGAGCCGT    720
TGCTGGGATT AATTGGGAGA GGAAGACAGC ATGTCGTTCG TGACCACACA GCCGGAAGCC    780
CTGGCAGCTG CGGCGGCGAA CCTACAGGGT ATTGGCACGA CAATGAACGC CCAGAACGCG    840
GCCGCGGCTG CTCCAACCAC CGGAGTAGTG CCCGCAGCCG CCGATGAAGT ATCAGCGCTG    900
ACCGCGGCTC AGTTTGCTGC GCACGCGCAG ATGTACCAAA CGGTCAGCGC CCAGGCCGCG    960
GCCATTCACG AAATGTTCGT GAACACGCTG GTGGCCAGTT CTGGCTCATA CGCGGCCACC   1020
GAGGCGGCCA ACGCAGCCGC TGCCGGCTGA ACGGGCTCGC ACGAACCTGC TGAAGGAGAG   1080
GGGGAACATC CGGAGTTCTC GGGTCAGGGG TTGCGCCAGC GCCCAGCCGA TTCAGNTATC   1140
GGCGTCCATA ACAGCAGACG ATCTAGGCAT TCAGTACTAA GGAGACAGGC AACATGGCCT   1200
CACGTTTTAT GACGGATCCG CATGCGATGC GGGACATGGC GGGCCGTTTT GAGGTGCACG   1260
CCCAGACGGT GGAGGACGAG GCTCGCCGGA TGTGGGCGTC CGCGCAAAAC ATTTCCGGTG   1320
CGGGCTGGAG TGGCATGGCC GAGGCGACCT CGCTAGACAC CATGACCTAG ATGAATCAGG   1380
CGTTTCGCAA CATCGTGAAC ATGCTGCACG GGGTGCGTGA CGGGCTGGTT CGCGACGCCA   1440
ACAANTACGA ACAGCAAGAG CAGGCCTCCC AGCAGATCCT GAGCAGNTAG CGCCGAAAGC   1500
CACAGCTGNG TACGNTTTCT CACATTAGGA GAACACCAAT ATGACGATTA ATTACCAGTT   1560
CGGGGACGTC GACGCTCATG GCGCCATGAT CCGCGCTCAG GCGGCGTCGC TTGAGGCGGA   1620
GCATCAGGCC ATCGTTCGTG ATGTGTTGGC CGCGGGTGAC TTTTGGGGCG GCGCCGGTTC   1680
GGTGGCTTGC CAGGAGTTCA TTACCCAGTT GGGCCGTAAC TTCCAGGTGA TCTACGAGCA   1740
GG                                                                  1742
```

(2) INFORMATION FOR SEQ ID NO:26: MTI (MTB9.9A)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2836 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Mycobacterium* tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
GTTGATTCCG TTCGCGGCGC CGCCGAAGAC CACCAACTCC GCTGGGGTGG TCGCACAGGC    60
GGTTGCGTCG GTCAGCTGGC CGAATCCCAA TGATTGGTGG CTCNGTGCGG TTGCTGGGCT   120
CGATTACCCC CACGGAAAGG ACGACGATCG TTCGTTTGCT CGGTCAGTCG TACTTGGCGA   180
CGGGCATGGC GCGGTTTCTT ACCTCGATCG CACAGCAGCT GACCTTCGGC CCAGGGGGCA   240
CAACGGCTGG CTCCGGCGGA GCCTGGTACC CAACGCCACA ATTCGCCGGC CTGGGTGCAG   300
GCCCGGCGGT GTCGGCGAGT TTGGCGCGGG CGGAGCCGGT CGGGAGGTTG TCGGTGCCGC   360
CAAGTTGGGC CGTCGCGGCT CCGGCCTTCG CGGAGAAGCC TGAGGCGGGC ACGCCGATGT   420
CCGTCATCGG CGAAGCGTCC AGCTGCGGTC AGGGAGGCCT GCTTCGAGGC ATACCGCTGG   480
CGAGAGCGGG GCGGCGTACA GGCGCCTTCG CTCACCGATA CGGGTTCCGC CACAGCGTGA   540
TTACCCGGTC TCCGTCGGCG GGATAGCTTT CGATCCGGTC TGCGCGGCCG CCGGAAATGC   600
TGCAGATAGC GATCGACCGC GCCGGTCGGT AAACGCCGCA CACGGCACTA TCAATGCGCA   660
CGGCGGGCGT TGATGCCAAA TTGACCGTCC CGACGGGGCT TTATCTGCGG CAAGATTTCA   720
TCCCCAGCCC GGTCGGTGGG CCGATAAATA CGCTGGTCAG CGCGACTCTT CCGGCTGAAT   780
TCGATGCTCT GGGCGCCCGC TCGACGCCGA GTATCTCGAG TGGGCCGCAA ACCCGGTCAA   840
ACGCTGTTAC TGTGGCGTTA CCACAGGTGA ATTTGCGGTG CCAACTGGTG AACACTTGCG   900
AACGGGTGGC ATCGAAATCA ACTTGTTGCG TTGCAGTGAT CTACTCTCTT GCAGAGAGCC   960
GTTGCTGGGA TTAATTGGGA GAGGAAGACA GCATGTCGTT CGTGACCACA CAGCCGGAAG  1020
CCCTGGCAGC TGCGGCGGCG AACCTACAGG GTATTGGCAC GACAATGAAC GCCCAGAACG  1080
CGGCCGCGGC TGCTCCAACC ACCGGAGTAG TGCCCGCAGC CGCCGATGAA GTATCAGCGC  1140
TGACCGCGGC TCAGTTTGCT GCGCACGCGC AGATGTACCA AACGGTCAGC GCCCAGGCCG  1200
CGGCCATTCA CGAAATGTTC GTGAACACGC TGGTGGCCAG TTCTGGCTCA TACGCGGCCA  1260
CCGAGGCGGC CAACGCAGCC GCTGCCGGCT GAACGGGCTC GCACGAACCT GCTGAAGGAG  1320
AGGGGGAACA TCCGGAGTTC TCGGGTCAGG GGTTGCGCCA GCGCCCAGCC GATTCAGCTA  1380
TCGGCGTCCA TAACAGCAGA CGATCTAGGC ATTCAGTACT AAGGAGACAG GCAACATGGC  1440
CTCACGTTTT ATGACGGATC CGCATGCGAT GCGGGACATG GCGGGCCGTT TTGAGGTGCA  1500
CGCCCAGACG GTGGAGGACG AGGCTCGCCG GATGTGGGCG TCCGCGCAAA ACATTTCCGG  1560
TGCGGGCTGG AGTGGCATGG CCGAGGCGAC CTCGCTAGAC ACCATGACCT AGATGAATCA  1620
GGCGTTTCGC AACATCGTGA ACATGCTGCA CGGGGTGCGT GACGGGCTGG TTCGCGACGC  1680
CAACAACTAC GAACAGCAAG AGCAGGCCTC CCAGCAGATC CTGAGCAGCT AGCGCCGAAA  1740
GCCACAGCTG CGTACGCTTT CTCACATTAG GAGAACACCA ATATGACGAT TAATTACCAG  1800
TTCGGGGACG TCGACGCTCA TGGCGCCATG ATCCGCGCTC AGGCGGCGTC GCTTGAGGCG  1860
GAGCATCAGG CCATCGTTCG TGATGTGTTG GCCGCGGGTG ACTTTTGGGG CGGCGCCGGT  1920
TCGGTGGCTT GCCAGGAGTT CATTACCCAG TTGGGCCGTA ACTTCCAGGT GATCTACGAG  1980
CAGGCCAACG CCCACGGGCA GAAGGTGCAG GCTGCCGGCA ACAACATGGC GCAAACCGAC  2040
AGCGCCGTCG GCTCCAGCTG GGCCTAAAAC TGAACTTCAG TCGCGGCAGC ACACCAACCA  2100
GCCGGTGTGC TGCTGTGTCC TGCAGTTAAC TAGCACTCGA CCGCTGAGGT AGCGATGGAT  2160
CAACAGAGTA CCCGCACCGA CATCACCGTC AACGTCGACG GCTTCTGGAT GCTTCAGGCG  2220
CTACTGGATA TCCGCCACGT TGCGCCTGAG TTACGTTGCC GGCCGTACGT CTCCACCGAT  2280
TCCAATGACT GGCTAAACGA GCACCCGGGG ATGGCGGTCA TGCGCGAGCA GGGCATTGTC  2340
GTCAACGACG CGGTCAACGA ACAGGTCGCT GCCCGGATGA AGGTGCTTGC CGCACCTGAT  2400
CTTGAAGTCG TCGCCCTGCT GTCACGCGGC AAGTTGCTGT ACGGGGTCAT AGACGACGAG  2460
AACCAGCCGC CGGGTTCGCG TGACATCCCT GACAATGAGT TCCGGGTGGT GTTGGCCCGG  2520
CGAGGCCAGC ACTGGGTGTC GGCGGTACGG GTTGGCAATG ACATCACCGT CGATGACGTG  2580
ACGGTCTCGG ATAGCGCCTC GATCGCCGCA CTGGTAATGG ACGGTCTGGA GTCGATTCAC  2640
CACGCCGACC CAGCCGCGAT CAACGCGGTC AACGTGCCAA TGGAGGAGAT CTCGTGCCGA  2700
ATTCGGCACG AGGCACGAGG CGGTGTCGGT GACGACGGGA TCGATCACGA TCATCGACCG  2760
GCCGGGATCC TTGGCGATCT CGTTGAGCAC GACCCGGGCC CGCGGGAAGC TCTGCGACAT  2820
CCATGGGTTC TTCCCG                                                  2836
```

(2) INFORMATION FOR SEQ ID NO:27: MTI (MTB9.9A)

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Mycobacterium* tuberculosis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
Met Thr Ile Asn Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met
1               5                   10                  15
Ile Arg Ala Leu Ala Gly Leu Leu Glu Ala Glu His Gln Ala Ile Ile
            20                  25                  30
Ser Asp Val Leu Thr Ala Ser Asp Phe Trp Gly Gly Ala Gly Ser Ala
        35                  40                  45
Ala Cys Gln Gly Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile
    50                  55                  60
Tyr Glu Gln Ala Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn
65                  70                  75                  80
Asn Met Ala Gln Thr Asp Ser Ala Val Gly Ser Ser Trp Ala
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:28: HTCC#1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
CAGGCATGAG CAGAGCGTTC ATCATCGATC CAACGATCAG TGCCATTGAC GGCTTGTACG     60
ACCTTCTGGG GATTGGAATA CCCAACCAAG GGGGTATCCT TTACTCCTCA CTAGAGTACT    120
TCGAAAAAGC CCTGGAGGAG CTGGCAGCAG CGTTTCCGGG TGATGGCTGG TTAGGTTCGG    180
CCGCGGACAA ATACGCCGGC AAAAACCGCA ACCACGTGAA TTTTTTCCAG GAACTGGCAG    240
ACCTCGATCG TCAGCTCATC AGCCTGATCC ACGACCAGGC CAACGCGGTC CAGACGACCC    300
GCGACATCCT GGAGGGCGCC AAGAAAGGTC TCGAGTTCGT GCGCCCGGTG GCTGTGGACC    360
TGACCTACAT CCCGGTCGTC GGGCACGCCC TATCGGCCGC CTTCCAGGCG CCGTTTTGCG    420
CGGGCGCGAT GGCCGTAGTG GGCGGCGCGC TTGCCTACTT GGTCGTGAAA ACGCTGATCA    480
ACGCGACTCA ACTCCTCAAA TTGCTTGCCA AATTGGCGGA GTTGGTCGCG GCCGCCATTG    540
CGGACATCAT TTCGGATGTG GCGGACATCA TCAAGGGCAC CCTCGGAGAA GTGTGGGAGT    600
TCATCACAAA CGCGCTCAAC GGCCTGAAAG AGCTTTGGGA CAAGCTCACG GGGTGGGTGA    660
CCGGACTGTT CTCTCGAGGG TGGTCGAACC TGGAGTCCTT CTTTGCGGGC GTCCCCGGCT    720
TGACCGGCGC GACCAGCGGC TTGTCGCAAG TGACTGGCTT GTTCGGTGCG GCCGGTCTGT    780
CCGCATCGTC GGGCTTGGCT CACGCGGATA GCCTGGCGAG CTCAGCCAGC TTGCCCGCCC    840
TGGCCGGCAT TGGGGGCGGG TCCGGTTTTG GGGGCTTGCC GAGCCTGGCT CAGGTCCATG    900
CCGCCTCAAC TCGGCAGGCG CTACGGCCCC GAGCTGATGG CCCGGTCGGC GCCGCTGCCG    960
AGCAGGTCGG CGGGCAGTCG CAGCTGGTCT CCGCGCAGGG TTCCCAAGGT ATGGGCGGAC   1020
CCGTAGGCAT GGGCGGCATG CACCCCTCTT CGGGGGCGTC GAAAGGGACG ACGACGAAGA   1080
AGTACTCGGA AGGCGCGGCG GCGGGCACTG AAGACGCCGA GCGCGCGCCA GTCGAAGCTG   1140
ACGCGGGCGG TGGGCAAAAG GTGCTGGTAC GAAACGTCGT CTAACGGCAT GGCGAGCCAA   1200
```

(2) INFORMATION FOR SEQ ID NO:29: HTCC#1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 392 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
Met Ser Arg Ala Phe Ile Ile Asp Pro Thr Ile Ser Ala Ile Asp Gly
```

```
1               5                   10                  15
Leu Tyr Asp Leu Leu Gly Ile Gly Ile Pro Asn Gln Gly Gly Ile Leu
            20                  25                  30
Tyr Ser Ser Leu Glu Tyr Phe Glu Lys Ala Leu Glu Glu Leu Ala Ala
        35                  40                  45
Ala Phe Pro Gly Asp Gly Trp Leu Gly Ser Ala Ala Asp Lys Tyr Ala
    50                  55                  60
Gly Lys Asn Arg Asn His Val Asn Phe Phe Gln Glu Leu Ala Asp Leu
65                  70                  75                  80
Asp Arg Gln Leu Ile Ser Leu Ile His Asp Gln Ala Asn Ala Val Gln
                85                  90                  95
Thr Thr Arg Asp Ile Leu Glu Gly Ala Lys Lys Gly Leu Glu Phe Val
            100                 105                 110
Arg Pro Val Ala Val Asp Leu Thr Tyr Ile Pro Val Val Gly His Ala
        115                 120                 125
Leu Ser Ala Ala Phe Gln Ala Pro Phe Cys Ala Gly Ala Met Ala Val
    130                 135                 140
Val Gly Gly Ala Leu Ala Tyr Leu Val Val Lys Thr Leu Ile Asn Ala
145                 150                 155                 160
Thr Gln Leu Leu Lys Leu Leu Ala Lys Leu Ala Glu Leu Val Ala Ala
                165                 170                 175
Ala Ile Ala Asp Ile Ile Ser Asp Val Ala Asp Ile Ile Lys Gly Thr
            180                 185                 190
Leu Gly Glu Val Trp Glu Phe Ile Thr Asn Ala Leu Asn Gly Leu Lys
        195                 200                 205
Glu Leu Trp Asp Lys Leu Thr Gly Trp Val Thr Gly Leu Phe Ser Arg
    210                 215                 220
Gly Trp Ser Asn Leu Glu Ser Phe Phe Ala Gly Val Pro Gly Leu Thr
225                 230                 235                 240
Gly Ala Thr Ser Gly Leu Ser Gln Val Thr Gly Leu Phe Gly Ala Ala
                245                 250                 255
Gly Leu Ser Ala Ser Ser Gly Leu Ala His Ala Asp Ser Leu Ala Ser
            260                 265                 270
Ser Ala Ser Leu Pro Ala Leu Ala Gly Ile Gly Gly Gly Ser Gly Phe
        275                 280                 285
Gly Gly Leu Pro Ser Leu Ala Gln Val His Ala Ala Ser Thr Arg Gln
    290                 295                 300
Ala Leu Arg Pro Arg Ala Asp Gly Pro Val Gly Ala Ala Ala Glu Gln
305                 310                 315                 320
Val Gly Gly Gln Ser Gln Leu Val Ser Ala Gln Gly Ser Gln Gly Met
                325                 330                 335
Gly Gly Pro Val Gly Met Gly Gly Met His Pro Ser Ser Gly Ala Ser
            340                 345                 350
Lys Gly Thr Thr Thr Lys Lys Tyr Ser Glu Gly Ala Ala Ala Gly Thr
        355                 360                 365
Glu Asp Ala Glu Arg Ala Pro Val Glu Ala Asp Ala Gly Gly Gly Gln
    370                 375                 380
Lys Val Leu Val Arg Asn Val Val
385                 390
```

(2) INFORMATION FOR SEQ ID NO:30: MTCC#2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1441 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
GAGGTTGCTG GCAATGGATT TCGGGCTTTT ACCTCCGGAA GTGAATTCAA GCCGAATGTA    60
TTCCGGTCCG GGGCCGGAGT CGATGCTAGC CGCCGCGGCC GCCTGGGACG GTGTGGCCGC   120
GGAGTTGACT TCCGCCGCGG TCTCGTATGG ATCGGTGGTG TCGACGCTGA TCGTTGAGCC   180
GTGGATGGGG CCGGCGGCGG CCGCGATGGC GGCCGCGGCA ACGCCGTATG TGGGGTGGCT   240
GGCCGCCACG GCGGCGCTGG CGAAGGAGAC GGCCACACAG GCGAGGGCAG CGGCGGAAGC   300
```

```
GTTTGGGACG GCGTTCGCGA TGACGGTGCC ACCATCCCTC GTCGCGGCCA ACCGCAGCCG     360
GTTGATGTCG CTGGTCGCGG CGAACATTCT GGGGCAAAAC AGTGCGGCGA TCGCGGCTAC     420
CCAGGCCGAG TATGCCGAAA TGTGGGCCCA AGACGCTGCC GTGATGTACA GCTATGAGGG     480
GGCATCTGCG GCCGCGTCGG CGTTGCCGCC GTTCACTCCA CCCGTGCAAG GCACCGGCCC     540
GGCCGGGCCC GCGGCCGCAG CCGCGGCGAC CCAAGCCGCC GGTGCGGGCG CCGTTGCGGA     600
TGCACAGGCG ACACTGGCCC AGCTGCCCCC GGGGATCCTG AGCGACATTC TGTCCGCATT     660
GGCCGCCAAC GCTGATCCGC TGACATCGGG ACTGTTGGGG ATCGCGTCGA CCCTCAACCC     720
GCAAGTCGGA TCCGCTCAGC CGATAGTGAT CCCCACCCCG ATAGGGGAAT TGGACGTGAT     780
CGCGCTCTAC ATTGCATCCA TCGCGACCGG CAGCATTGCG CTCGCGATCA CGAACACGGC     840
CAGACCCTGG CACATCGGCC TATACGGGAA CGCCGGCGGG CTGGGACCGA CGCAGGGCCA     900
TCCACTGAGT TCGGCGACCG ACGAGCCGGA GCCGCACTGG GGCCCCTTCG GGGGCGCGGC     960
GCCGGTGTCC GCGGGCGTCG GCCACGCAGC ATTAGTCGGA GCGTTGTCGG TGCCGCACAG    1020
CTGGACCACG GCCGCCCCGG AGATCCAGCT CGCCGTTCAG GCAACACCCA CCTTCAGCTC    1080
CAGCGCCGGC GCCGACCCGA CGGCCCTAAA CGGGATGCCG GCAGGCCTGC TCAGCGGGAT    1140
GGCTTTGGCG AGCCTGGCCG CACGCGGCAC GACGGGCGGT GGCGGCACCC GTAGCGGCAC    1200
CAGCACTGAC GGCCAAGAGG ACGGCCGCAA ACCCCCGGTA GTTGTGATTA GAGAGCAGCC    1260
GCCGCCCGGA AACCCCCCGC GGTAAAAGTC CGGCAACCGT TCGTCGCCGC GCGGAAAATG    1320
CCTGGTGAGC GTGGCTATCC GACGGGCCGT TCACACCGCT TGTAGTAGCG TACGGCTATG    1380
GACGACGGTG TCTGGATTCT CGGCGGCTAT CAGAGCGATT TTGCTCGCAA CCTCAGCAAA    1440
G                                                                   1441
```

(2) INFORMATION FOR SEQ ID NO:31: MTCC#2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 423 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

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

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

(2) INFORMATION FOR SEQ ID NO:32: ESAT-6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 154 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
ATGACAGAGC AGCAGTGGAA TTTCGCGGGT ATCGAGGCCG CGGCAAGCGC AATCCAGGGA    60
AATGTCACGT CCATTCATTC CCTCCTTGAC GAGGGGAAGC AGTCCCTGAC CAAGCTCGCA   120
GCGGCCTGGG GCGGTAGCGG TTCGGAAGCG TACC                               154
```

(2) INFORMATION FOR SEQ ID NO:33: ESAT-6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Met Thr Glu Gln Gln Trp Asn Phe Ala Gly Ile Glu Ala Ala Ala Ser
1               5                   10                  15
Ala Ile Gln Gly Asn Val Thr Ser Ile His Ser Leu Leu Asp Glu Gly
            20                  25                  30
Lys Gln Ser Leu Thr Lys Leu Ala Ala Ala Trp Gly Gly Ser Gly Ser
        35                  40                  45
Glu Ala Tyr
    50
```

(2) INFORMATION FOR SEQ ID NO:34: Tb38-1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
CGGCACGAGA GACCGATGCC GCTACCCTCG CGCAGGAGGC AGGTAATTTC GAGCGGATCT    60
```

```
CCGGCGACCT GAAAACCCAG ATCGACCAGG TGGAGTCGAC GGCAGGTTCG TTGCAGGGCC     120
AGTGGCGCGG CGCGGCGGGG ACGGCCGCCC AGGCCGCGGT GGTGCGCTTC CAAGAAGCAG     180
CCAATAAGCA GAAGCAGGAA CTCGACGAGA TCTCGACGAA TATTCGTCAG GCCGGCGTCC     240
AATACTCGAG GGCCGACGAG GAGCAGCAGC AGGCGCTGTC CTCGCAAATG GGCTTCTGAC     300
CCGCTAATAC GAAAAGAAAC GGAGCAA                                         327
```

(2) INFORMATION FOR SEQ ID NO:35: Tb38-1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Thr Asp Ala Ala Thr Leu Ala Gln Glu Ala Gly Asn Phe Glu Arg Ile
1               5                   10                  15
Ser Gly Asp Leu Lys Thr Gln Ile Asp Gln Val Glu Ser Thr Ala Gly
            20                  25                  30
Ser Leu Gln Gly Gln Trp Arg Gly Ala Ala Gly Thr Ala Ala Gln Ala
        35                  40                  45
Ala Val Val Arg Phe Gln Glu Ala Ala Asn Lys Gln Lys Gln Glu Leu
    50                  55                  60
Asp Glu Ile Ser Thr Asn Ile Arg Gln Ala Gly Val Gln Tyr Ser Arg
65                  70                  75                  80
Ala Asp Glu Glu Gln Gln Gln Ala Leu Ser Ser Gln Met Gly Phe
            85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:36: TbRa3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
GAATTCGGCA CGAGAGGTGA TCGACATCAT CGGGACCAGC CCCACATCCT GGGAACAGGC      60
GGCGGCGGAG GCGGTCCAGC GGGCGCGGGA TAGCGTCGAT GACATCCGCG TCGCTCGGGT     120
CATTGAGCAG GACATGGCCG TGGACAGCGC CGGCAAGATC ACCTACCGCA TCAAGCTCGA     180
AGTGTCGTTC AAGATGAGGC CGGCGCAACC GCGCTAGCAC GGGCCGGCGA GCAAGACGCA     240
AAATCGCACG GTTTGCGGTT GATTCGTGCG ATTTTGTGTC TGCTCGCCGA GGCCTACCAG     300
GCGCGGCCCA GGTCCGCGTG CTGCCGTATC CAGGCGTGCA TCGCGATTCC GGCGGCCACG     360
CCGGAGTTAA TGCTTCGCGT CGACCCGAAC TGGGCGATCC GCCGGNGAGC TGATCGATGA     420
CCGTGGCCAG CCCGTCGATG CCCGAGTTGC CCGAGGAAAC GTGCTGCCAG GCCGGTAGGA     480
AGCGTCCGTA GGCGGCGGTG CTGACCGGCT CTGCCTGCGC CCTCAGTGCG GCCAGCGAGC     540
GG                                                                    542
```

(2) INFORMATION FOR SEQ ID NO:37: TbRa3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Val Ile Asp Ile Ile Gly Thr Ser Pro Thr Ser Trp Glu Gln Ala Ala
1               5                   10                  15
Ala Glu Ala Val Gln Arg Ala Arg Asp Ser Val Asp Asp Ile Arg Val
            20                  25                  30
Ala Arg Val Ile Glu Gln Asp Met Ala Val Asp Ser Ala Gly Lys Ile
```

```
            35                  40                  45
    Thr Tyr Arg Ile Lys Leu Glu Val Ser Phe Lys Met Arg Pro Ala Gln
        50                  55                  60
    Pro Arg
    65
```

(2) INFORMATION FOR SEQ ID NO:38: 38 kD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1993 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA     60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC    120
GCGGAAATTG AAGAGCACAG AAAGGTATGG CGTGAAAATT CGTTTGCATA CGCTGTTGGC    180
CGTGTTGACC GCTGCGCCGC TGCTGCTAGC AGCGGCGGGC TGTGGCTCGA AACCACCGAG    240
CGGTTCGCCT GAAACGGGCG CCGGCGCCGG TACTGTCGCG ACTACCCCCG CGTCGTCGCC    300
GGTGACGTTG GCGGAGACCG GTAGCACGCT GCTCTACCCG CTGTTCAACC TGTGGGGTCC    360
GGCCTTTCAC GAGAGGTATC CGAACGTCAC GATCACCGCT CAGGGCACCG GTTCTGGTGC    420
CGGGATCGCG CAGGCCGCCG CCGGGACGGT CAACATTGGG GCCTCCGACG CCTATCTGTC    480
GGAAGGTGAT ATGGCCGCGC ACAAGGGGCT GATGAACATC GCGCTAGCCA TCTCCGCTCA    540
GCAGGTCAAC TACAACCTGC CCGGAGTGAG CGAGCACCTC AAGCTGAACG GAAAAGTCCT    600
GGCGGCCATG TACCAGGGCA CCATCAAAAC CTGGGACGAC CCGCAGATCG CTGCGCTCAA    660
CCCCGGCGTG AACCTGCCCG GCACCGCGGT AGTTCCGCTG CACCGCTCCG ACGGGTCCGG    720
TGACACCTTC TTGTTCACCC AGTACCTGTC CAAGCAAGAT CCCGAGGGCT GGGGCAAGTC    780
GCCCGGCTTC GGCACCACCG TCGACTTCCC GGCGGTGCCG GGTGCGCTGG GTGAGAACGG    840
CAACGGCGGC ATGGTGACCG GTTGCGCCGA GACACCGGGC TGCGTGGCCT ATATCGGCAT    900
CAGCTTCCTC GACCAGGCCA GTCAACGGGG ACTCGGCGAG GCCCAACTAG GCAATAGCTC    960
TGGCAATTTC TTGTTGCCCG ACGCGCAAAG CATTCAGGCC GCGGCGGCTG GCTTCGCATC   1020
GAAAACCCCG GCGAACCAGG CGATTTCGAT GATCGACGGG CCCGCCCCGG ACGGCTACCC   1080
GATCATCAAC TACGAGTACG CCATCGTCAA CAACCGGCAA AAGGACGCCG CCACCGCGCA   1140
GACCTTGCAG GCATTTCTGC ACTGGGCGAT CACCGACGGC AACAAGGCCT CGTTCCTCGA   1200
CCAGGTTCAT TTCCAGCCGC TGCCGCCCGC GGTGGTGAAG TTGTCTGACG CGTTGATCGC   1260
GACGATTTCC AGCTAGCCTC GTTGACCACC ACGCGACAGC AACCTCCGTC GGGCCATCGG   1320
GCTGCTTTGC GGAGCATGCT GGCCCGTGCC GGTGAAGTCG GCCGCGCTGG CCCGGCCATC   1380
CGGTGGTTGG GTGGGATAGG TGCGGTGATC CCGCTGCTTG CGCTGGTCTT GGTGCTGGTG   1440
GTGCTGGTCA TCGAGGCGAT GGGTGCGATC AGGCTCAACG GGTTGCATTT CTTCACCGCC   1500
ACCGAATGGA ATCCAGGCAA CACCTACGGC GAAACCGTTG TCACCGACGC GTCGCCCATC   1560
```

```
CGGTCGGCGC CTACTACGGG GCGTTGCCGC TGATCGTCGG GACGCTGGCG ACCTCGGCAA    1620

TCGCCCTGAT CATCGCGGTG CCGGTCTCTG TAGGAGCGGC GCTGGTGATC GTGGAACGGC    1680

TGCCGAAACG GTTGGCCGAG GCTGTGGGAA TAGTCCTGGA ATTGCTCGCC GGAATCCCCA    1740

GCGTGGTCGT CGGTTTGTGG GGGGCAATGA CGTTCGGGCC GTTCATCGCT CATCACATCG    1800

CTCCGGTGAT CGCTCACAAC GCTCCCGATG TGCCGGTGCT GAACTACTTG CGCGGCGACC    1860

CGGGCAACGG GGAGGGCATG TTGGTGTCCG GTCTGGTGTT GGCGGTGATG GTCGTTCCCA    1920

TTATCGCCAC CACCACTCAT GACCTGTTCC GGCAGGTGCC GGTGTTGCCC CGGGAGGGCG    1980

CGATCGGGAA TTC                                                       1993
```

(2) INFORMATION FOR SEQ ID NO:39: 38 kD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 374 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

```
Met Lys Ile Arg Leu His Thr Leu Leu Ala Val Leu Thr Ala Ala Pro
1               5                   10                  15

Leu Leu Leu Ala Ala Ala Gly Cys Gly Ser Lys Pro Pro Ser Gly Ser
            20                  25                  30

Pro Glu Thr Gly Ala Gly Ala Gly Thr Val Ala Thr Thr Pro Ala Ser
        35                  40                  45

Ser Pro Val Thr Leu Ala Glu Thr Gly Ser Thr Leu Leu Tyr Pro Leu
    50                  55                  60

Phe Asn Leu Trp Gly Pro Ala Phe His Glu Arg Tyr Pro Asn Val Thr
65                  70                  75                  80

Ile Thr Ala Gln Gly Thr Gly Ser Gly Ala Gly Ile Ala Gln Ala Ala
                85                  90                  95

Ala Gly Thr Val Asn Ile Gly Ala Ser Asp Ala Tyr Leu Ser Glu Gly
            100                 105                 110

Asp Met Ala Ala His Lys Gly Leu Met Asn Ile Ala Leu Ala Ile Ser
        115                 120                 125

Ala Gln Gln Val Asn Tyr Asn Leu Pro Gly Val Ser Glu His Leu Lys
    130                 135                 140

Leu Asn Gly Lys Val Leu Ala Ala Met Tyr Gln Gly Thr Ile Lys Thr
145                 150                 155                 160

Trp Asp Asp Pro Gln Ile Ala Ala Leu Asn Pro Gly Val Asn Leu Pro
                165                 170                 175

Gly Thr Ala Val Val Pro Leu His Arg Ser Asp Gly Ser Gly Asp Thr
            180                 185                 190

Phe Leu Phe Thr Gln Tyr Leu Ser Lys Gln Asp Pro Glu Gly Trp Gly
        195                 200                 205

Lys Ser Pro Gly Phe Gly Thr Thr Val Asp Phe Pro Ala Val Pro Gly
```

```
        210                 215                 220

    Ala Leu Gly Glu Asn Gly Asn Gly Gly Met Val Thr Gly Cys Ala Glu
    225                 230                 235                 240

    Thr Pro Gly Cys Val Ala Tyr Ile Gly Ile Ser Phe Leu Asp Gln Ala
                    245                 250                 255

    Ser Gln Arg Gly Leu Gly Glu Ala Gln Leu Gly Asn Ser Ser Gly Asn
                260                 265                 270

    Phe Leu Leu Pro Asp Ala Gln Ser Ile Gln Ala Ala Ala Ala Gly Phe
            275                 280                 285

    Ala Ser Lys Thr Pro Ala Asn Gln Ala Ile Ser Met Ile Asp Gly Pro
        290                 295                 300

    Ala Pro Asp Gly Tyr Pro Ile Ile Asn Tyr Glu Tyr Ala Ile Val Asn
    305                 310                 315                 320

    Asn Arg Gln Lys Asp Ala Ala Thr Ala Gln Thr Leu Gln Ala Phe Leu
                    325                 330                 335

    His Trp Ala Ile Thr Asp Gly Asn Lys Ala Ser Phe Leu Asp Gln Val
                340                 345                 350

    His Phe Gln Pro Leu Pro Pro Ala Val Val Lys Leu Ser Asp Ala Leu
            355                 360                 365

    Ile Ala Thr Ile Ser Ser
        370
```

(2) INFORMATION FOR SEQ ID NO:40: DPEP

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 999 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

```
ATGCATCACC ATCACCATCA CATGCATCAG GTGGACCCCA ACTTGACACG TCGCAAGGGA     60
CGATTGGCGG CACTGGCTAT CGCGGCGATG GCCAGCGCCA GCCTGGTGAC CGTTGCGGTG    120
CCCGCGACCG CCAACGCCGA TCCGGAGCCA GCGCCCCCGG TACCCACAAC GGCCGCCTCG    180
CCGCCGTCGA CCGCTGCAGC GCCACCCGCA CCGGCGACAC CTGTTGCCCC CCCACCACCG    240
GCCGCCGCCA ACACGCCGAA TGCCCAGCCG GGCGATCCCA ACGCAGCACC TCCGCCGGCC    300
GACCCGAACG CACCGCCGCC ACCTGTCATT GCCCCAAACG CACCCCAACC TGTCCGGATC    360
GACAACCCGG TTGGAGGATT CAGCTTCGCG CTGCCTGCTG GCTGGGTGGA GTCTGACGCC    420
GCCCACTTCG ACTACGGTTC AGCACTCCTC AGCAAAACCA CCGGGGACCC GCCATTTCCC    480
GGACAGCCGC CGCCGGTGGC CAATGACACC CGTATCGTGC TCGGCCGGCT AGACCAAAAG    540
CTTTACGCCA GCGCCGAAGC CACCGACTCC AAGGCCGCGG CCCGGTTGGG CTCGGACATG    600
GGTGAGTTCT ATATGCCCTA CCCGGGCACC CGGATCAACC AGGAAACCGT CTCGCTCGAC    660
GCCAACGGGG TGTCTGGAAG CGCGTCGTAT TACGAAGTCA AGTTCAGCGA TCCGAGTAAG    720
CCGAACGGCC AGATCTGGAC GGGCGTAATC GGCTCGCCCG CGGCGAACGC ACCGGACGCC    780
GGGCCCCCTC AGCGCTGGTT TGTGGTATGG CTCGGGACCG CCAACAACCC GGTGGACAAG    840
GGCGCGGCCA AGGCGCTGGC CGAATCGATC CGGCCTTTGG TCGCCCCGCC GCCGGCGCCG    900
GCACCGGCTC CTGCAGAGCC CGCTCCGGCG CCGGCGCCGG CCGGGGAAGT CGCTCCTACC    960
CCGACGACAC CGACACCGCA GCGGACCTTA CCGGCCTGA                           999
```

(2) INFORMATION FOR SEQ ID NO:41: DPEP

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 332 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

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

(2) INFORMATION FOR SEQ ID NO:42: TbH4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 702 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
CGGCACGAGG ATCGGTACCC CGCGGCATCG GCAGCTGCCG ATTCGCCGGG TTTCCCCACC    60
CGAGGAAAGC CGCTACCAGA TGGCGCTGCC GAAGTAGGGC GATCCGTTCG CGATGCCGGC   120
ATGAACGGGC GGCATCAAAT TAGTGCAGGA ACCTTTCAGT TTAGCGACGA TAATGGCTAT   180
AGCACTAAGG AGGATGATCC GATATGACGC AGTCGCAGAC CGTGACGGTG GATCAGCAAG   240
AGATTTTGAA CAGGGCCAAC GAGGTGGAGG CCCCGATGGC GGACCCACCG ACTGATGTCC   300
CCATCACACC GTGCGAACTC ACGGNGGNTA AAAACGCCGC CCAACAGNTG GTNTTGTCCG   360
CCGACAACAT GCGGGAATAC CTGGCGGCCG GTGCCAAAGA GCGGCAGCGT CTGGCGACCT   420
CGCTGCGCAA CGCGGCCAAG GNGTATGGCG AGGTTGATGA GGAGGCTGCG ACCGCGCTGG   480
ACAACGACGG CGAAGGAACT GTGCAGGCAG AATCGGCCGG GGCCGTCGGA GGGGACAGTT   540
CGGCCGAACT AACCGATACG CCGAGGGTGG CCACGGCCGG TGAACCCAAC TTCATGGATC   600
```

```
TCAAAGAAGC GGCAAGGAAG CTCGAAACGG GCGACCAAGG CGCATCGCTC GCGCACTGNG    660
GGGATGGGTG GAACACTTNC ACCCTGACGC TGCAAGGCGA CG                       702
```

5 (2) INFORMATION FOR SEQ ID NO:43: TbH4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 286 amino acids
(B) TYPE: amino acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

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

(2) INFORMATION FOR SEQ ID NO:44: DPPD

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
60

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
65   ATGAAGTTGA AGTTTGCTCG CCTGAGTACT GCGATACTGG GTTGTGCAGC GGCGCTTGTG     60
     TTTCCTGCCT CGGTTGCCAG CGCAGATCCA CCTGACCCGC ATCAGCCGGA CATGACGAAA    120
     GGCTATTGCC CGGGTGGCCG ATGGGGTTTT GGCGACTTGG CCGTGTGCGA CGGCGAGAAG    180
     TACCCCGACG GCTCGTTTTG GCACCAGTGG ATGCAAACGT GGTTTACCGG CCCACAGTTT    240
```

```
TACTTCGATT GTGTCAGCGG CGGTGAGCCC CTCCCCGGCC CGCCGCCACC GGGTGGTTGC     300
GGTGGGGCAA TTCCGTCCGA GCAGCCCAAC GCTCCCTGA                            339
```

(2) INFORMATION FOR SEQ ID NO:45: DPPD

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Met Lys Leu Lys Phe Ala Arg Leu Ser Thr Ala Ile Leu Gly Cys Ala
 1               5                  10                  15
Ala Ala Leu Val Phe Pro Ala Ser Val Ala Ser Ala Asp Pro Pro Asp
            20                  25                  30
Pro His Gln Pro Asp Met Thr Lys Gly Tyr Cys Pro Gly Gly Arg Trp
        35                  40                  45
Gly Phe Gly Asp Leu Ala Val Cys Asp Gly Glu Lys Tyr Pro Asp Gly
    50                  55                  60
Ser Phe Trp His Gln Trp Met Gln Thr Trp Phe Thr Gly Pro Gln Phe
65                  70                  75                  80
Tyr Phe Asp Cys Val Ser Gly Gly Glu Pro Leu Pro Gly Pro Pro Pro
                85                  90                  95
Pro Gly Gly Cys Gly Gly Ala Ile Pro Ser Glu Gln Pro Asn Ala Pro
            100                 105                 110
```

```
<210> SEQ ID NO:46
<211> 921
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:tri-fusion
      protein DPV-MTI-MSL (designated Mtb31f)
<222> (1)..(900)

cat atg cat cac cat cac cat cac gat ccc gtg gac gcg gtc att aac    48
His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
 1               5                  10                  15

acc acc tgc aat tac ggg cag gta gta gct gcg ctc aac gcg acg gat    96
Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
            20                  25                  30

ccg ggg gct gcc gca cag ttc aac gcc tca ccg gtg gcg cag tcc tat    144
Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
        35                  40                  45

ttg cgc aat ttc ctc gcc gca ccg cca cct cag cgc gct gcc atg gcc    192
Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
    50                  55                  60

gcg caa ttg caa gct gtg ccg ggg gcg gca cag tac atc ggc ctt gtc    240
Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
 65                 70                  75                  80

gag tcg gtt gcc ggc tcc tgc aac aac tat gag ctc atg acg att aat    288
Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                85                  90                  95

tac cag ttc ggg gac gtc gac gct cat ggc gcc atg atc cgc gct cag    336
Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110
```

```
gcg gcg tcg ctt gag gcg gag cat cag gcc atc gtt cgt gat gtg ttg    384
Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

gcc gcg ggt gac ttt tgg ggc ggc gcc ggt tcg gtg gct tgc cag gag    432
Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

ttc att acc cag ttg ggc cgt aac ttc cag gtg atc tac gag cag gcc    480
Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

aac gcc cac ggg cag aag gtg cag gct gcc ggc aac aac atg gcg caa    528
Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

acc gac agc gcc gtc ggc tcc agc tgg gcc act agt atg agc ctt ttg    576
Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

gat gct cat atc cca cag ttg gtg gcc tcc cag tcg gcg ttt gcc gcc    624
Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

aag gcg ggg ctg atg cgg cac acg atc ggt cag gcc gag cag gcg gcg    672
Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

atg tcg gct cag gcg ttt cac cag ggg gag tcg tcg gcg gcg ttt cag    720
Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

gcc gcc cat gcc cgg ttt gtg gcg gcg gcc gcc aaa gtc aac acc ttg    768
Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys Val Asn Thr Leu
                245                 250                 255

ttg gat gtc gcg cag gcg aat ctg ggt gag gcc gcc ggt acc tat gtg    816
Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

gcc gcc gat gct gcg gcc gcg tcg acc tat acc ggg ttc gat atc cat    864
Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile His
        275                 280                 285

cac act ggc ggc cgc tcg agc aga tcc ggc tgc taacaaagcc cgaaaggaag 917
His Thr Gly Gly Arg Ser Ser Arg Ser Gly Cys
    290                 295

ctga                                                               921


&lt;210&gt; SEQ ID NO:47
&lt;211&gt; 299
&lt;212&gt; PRT
&lt;213&gt; Artificial Sequence
&lt;223&gt; Description of Artificial Sequence:tri-fusion
      protein DPV-MTI-MSL (designated Mtb31f)

His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
  1               5                  10                  15

Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
             20                  25                  30

Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
         35                  40                  45

Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
```

```
        50                  55                  60

Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
 65                  70                  75                  80

Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                85                  90                  95

Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110

Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

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

Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile His
        275                 280                 285

His Thr Gly Gly Arg Ser Ser Arg Ser Gly Cys
    290                 295


<210> SEQ ID NO:48
<211> 2168
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence:tetra-fusion
      protein DPV-MTI-MSL-MTCC2 (designated Mtb71f)
<222> (1)..(2133)

cat atg cat cac cat cac cat cac gat ccc gtg gac gcg gtc att aac     48
His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
  1               5                  10                  15

acc acc tgc aat tac ggg cag gta gta gct gcg ctc aac gcg acg gat     96
Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
            20                  25                  30

ccg ggg gct gcc gca cag ttc aac gcc tca ccg gtg gcg cag tcc tat    144
Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
        35                  40                  45
```

```
ttg cgc aat ttc ctc gcc gca ccg cca cct cag cgc gct gcc atg gcc     192
Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
     50                  55                  60

gcg caa ttg caa gct gtg ccg ggg gcg gca cag tac atc ggc ctt gtc     240
Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
65                  70                  75                  80

gag tcg gtt gcc ggc tcc tgc aac aac tat gag ctc atg acg att aat     288
Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                85                  90                  95

tac cag ttc ggg gac gtc gac gct cat ggc gcc atg atc cgc gct cag     336
Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110

gcg gcg tcg ctt gag gcg gag cat cag gcc atc gtt cgt gat gtg ttg     384
Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

gcc gcg ggt gac ttt tgg ggc ggc gcc ggt tcg gtg gct tgc cag gag     432
Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

ttc att acc cag ttg ggc cgt aac ttc cag gtg atc tac gag cag gcc     480
Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
145                 150                 155                 160

aac gcc cac ggg cag aag gtg cag gct gcc ggc aac aac atg gcg caa     528
Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
                165                 170                 175

acc gac agc gcc gtc ggc tcc agc tgg gcc act agt atg agc ctt ttg     576
Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
            180                 185                 190

gat gct cat atc cca cag ttg gtg gcc tcc cag tcg gcg ttt gcc gcc     624
Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
        195                 200                 205

aag gcg ggg ctg atg cgg cac acg atc ggt cag gcc gag cag gcg gcg     672
Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
    210                 215                 220

atg tcg gct cag gcg ttt cac cag ggg gag tcg tcg gcg gcg ttt cag     720
Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225                 230                 235                 240

gcc gcc cat gcc cgg ttt gtg gcg gcg gcc gcc aaa gtc aac acc ttg     768
Ala Ala His Ala Arg Phe Val Ala Ala Ala Ala Lys Val Asn Thr Leu
                245                 250                 255

ttg gat gtc gcg cag gcg aat ctg ggt gag gcc gcc ggt acc tat gtg     816
Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
            260                 265                 270

gcc gcc gat gct gcg gcc gcg tcg acc tat acc ggg ttc gat atc atg     864
Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile Met
        275                 280                 285

gat ttc ggg ctt tta cct ccg gaa gtg aat tca agc cga atg tat tcc     912
Asp Phe Gly Leu Leu Pro Pro Glu Val Asn Ser Ser Arg Met Tyr Ser
    290                 295                 300

ggt ccg ggg ccg gag tcg atg cta gcc gcc gcg gcc gcc tgg gac ggt     960
Gly Pro Gly Pro Glu Ser Met Leu Ala Ala Ala Ala Ala Trp Asp Gly
305                 310                 315                 320
```

```
gtg gcc gcg gag ttg act tcc gcc gcg gtc tcg tat gga tcg gtg gtg    1008
Val Ala Ala Glu Leu Thr Ser Ala Ala Val Ser Tyr Gly Ser Val Val
                325                 330                 335

tcg acg ctg atc gtt gag ccg tgg atg ggg ccg gcg gcg gcc gcg atg    1056
Ser Thr Leu Ile Val Glu Pro Trp Met Gly Pro Ala Ala Ala Ala Met
            340                 345                 350

gcg gcc gcg gca acg ccg tat gtg ggg tgg ctg gcc gcc acg gcg gcg    1104
Ala Ala Ala Ala Thr Pro Tyr Val Gly Trp Leu Ala Ala Thr Ala Ala
        355                 360                 365

ctg gcg aag gag acg gcc aca cag gcg agg gca gcg gcg gaa gcg ttt    1152
Leu Ala Lys Glu Thr Ala Thr Gln Ala Arg Ala Ala Ala Glu Ala Phe
    370                 375                 380

ggg acg gcg ttc gcg atg acg gtg cca cca tcc ctc gtc gcg gcc aac    1200
Gly Thr Ala Phe Ala Met Thr Val Pro Pro Ser Leu Val Ala Ala Asn
385                 390                 395                 400

cgc agc cgg ttg atg tcg ctg gtc gcg gcg aac att ctg ggg caa aac    1248
Arg Ser Arg Leu Met Ser Leu Val Ala Ala Asn Ile Leu Gly Gln Asn
                405                 410                 415

agt gcg gcg atc gcg gct acc cag gcc gag tat gcc gaa atg tgg gcc    1296
Ser Ala Ala Ile Ala Ala Thr Gln Ala Glu Tyr Ala Glu Met Trp Ala
            420                 425                 430

caa gac gct gcc gtg atg tac agc tat gag ggg gca tct gcg gcc gcg    1344
Gln Asp Ala Ala Val Met Tyr Ser Tyr Glu Gly Ala Ser Ala Ala Ala
        435                 440                 445

tcg gcg ttg ccg ccg ttc act cca ccc gtg caa ggc acc ggc ccg gcc    1392
Ser Ala Leu Pro Pro Phe Thr Pro Pro Val Gln Gly Thr Gly Pro Ala
    450                 455                 460

ggg ccc gcg gcc gca gcc gcg gcg acc caa gcc gcc ggt gcg ggc gcc    1440
Gly Pro Ala Ala Ala Ala Ala Ala Thr Gln Ala Ala Gly Ala Gly Ala
465                 470                 475                 480

gtt gcg gat gca cag gcg aca ctg gcc cag ctg ccc ccg ggg atc ctg    1488
Val Ala Asp Ala Gln Ala Thr Leu Ala Gln Leu Pro Pro Gly Ile Leu
                485                 490                 495

agc gac att ctg tcc gca ttg gcc gcc aac gct gat ccg ctg aca tcg    1536
Ser Asp Ile Leu Ser Ala Leu Ala Ala Asn Ala Asp Pro Leu Thr Ser
            500                 505                 510

gga ctg ttg ggg atc gcg tcg acc ctc aac ccg caa gtc gga tcc gct    1584
Gly Leu Leu Gly Ile Ala Ser Thr Leu Asn Pro Gln Val Gly Ser Ala
        515                 520                 525

cag ccg ata gtg atc ccc acc ccg ata ggg gaa ttg gac gtg atc gcg    1632
Gln Pro Ile Val Ile Pro Thr Pro Ile Gly Glu Leu Asp Val Ile Ala
    530                 535                 540

ctc tac att gca tcc atc gcg acc ggc agc att gcg ctc gcg atc acg    1680
Leu Tyr Ile Ala Ser Ile Ala Thr Gly Ser Ile Ala Leu Ala Ile Thr
545                 550                 555                 560

aac acg gcc aga ccc tgg cac atc ggc cta tac ggg aac gcc ggc ggg    1728
Asn Thr Ala Arg Pro Trp His Ile Gly Leu Tyr Gly Asn Ala Gly Gly
                565                 570                 575

ctg gga ccg acg cag ggc cat cca ctg agt tcg gcg acc gac gag ccg    1776
Leu Gly Pro Thr Gln Gly His Pro Leu Ser Ser Ala Thr Asp Glu Pro
            580                 585                 590
```

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gag ccg cac tgg ggc ccc ttc ggg ggc gcg gcg ccg gtg tcc gcg ggc    1824
Glu Pro His Trp Gly Pro Phe Gly Gly Ala Ala Pro Val Ser Ala Gly
        595                 600                 605

gtc ggc cac gca gca tta gtc gga gcg ttg tcg gtg ccg cac agc tgg    1872
Val Gly His Ala Ala Leu Val Gly Ala Leu Ser Val Pro His Ser Trp
    610                 615                 620

acc acg gcc gcc ccg gag atc cag ctc gcc gtt cag gca aca ccc acc    1920
Thr Thr Ala Ala Pro Glu Ile Gln Leu Ala Val Gln Ala Thr Pro Thr
625                 630                 635                 640

ttc agc tcc agc gcc ggc gcc gac ccg acg gcc cta aac ggg atg ccg    1968
Phe Ser Ser Ser Ala Gly Ala Asp Pro Thr Ala Leu Asn Gly Met Pro
                645                 650                 655

gca ggc ctg ctc agc ggg atg gct ttg gcg agc ctg gcc gca cgc ggc    2016
Ala Gly Leu Leu Ser Gly Met Ala Leu Ala Ser Leu Ala Ala Arg Gly
            660                 665                 670

acg acg ggc ggt ggc ggc acc cgt agc ggc acc agc act gac ggc caa    2064
Thr Thr Gly Gly Gly Gly Thr Arg Ser Gly Thr Ser Thr Asp Gly Gln
        675                 680                 685

gag gac ggc cgc aaa ccc ccg gta gtt gtg att aga gag cag ccg ccg    2112
Glu Asp Gly Arg Lys Pro Pro Val Val Val Ile Arg Glu Gln Pro Pro
    690                 695                 700

ccc gga aac ccc ccg cgg taagatttct aaatccatca cactggcggc cgctcgag 2168
Pro Gly Asn Pro Pro Arg
705                 710


<210> SEQ ID NO:49
<211> 710
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:tetra-fusion
      protein DPV-MTI-MSL-MTCC2 (designated Mtb71f)

His Met His His His His His His Asp Pro Val Asp Ala Val Ile Asn
  1               5                  10                  15

Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp
             20                  25                  30

Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr
         35                  40                  45

Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala
     50                  55                  60

Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val
 65                  70                  75                  80

Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr Glu Leu Met Thr Ile Asn
                 85                  90                  95

Tyr Gln Phe Gly Asp Val Asp Ala His Gly Ala Met Ile Arg Ala Gln
            100                 105                 110

Ala Ala Ser Leu Glu Ala Glu His Gln Ala Ile Val Arg Asp Val Leu
        115                 120                 125

Ala Ala Gly Asp Phe Trp Gly Gly Ala Gly Ser Val Ala Cys Gln Glu
    130                 135                 140

Phe Ile Thr Gln Leu Gly Arg Asn Phe Gln Val Ile Tyr Glu Gln Ala
```

145 150 155 160

Asn Ala His Gly Gln Lys Val Gln Ala Ala Gly Asn Asn Met Ala Gln
165 170 175

5

Thr Asp Ser Ala Val Gly Ser Ser Trp Ala Thr Ser Met Ser Leu Leu
180 185 190

Asp Ala His Ile Pro Gln Leu Val Ala Ser Gln Ser Ala Phe Ala Ala
10 195 200 205

Lys Ala Gly Leu Met Arg His Thr Ile Gly Gln Ala Glu Gln Ala Ala
210 215 220

15 Met Ser Ala Gln Ala Phe His Gln Gly Glu Ser Ser Ala Ala Phe Gln
225 230 235 240

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

20

Leu Asp Val Ala Gln Ala Asn Leu Gly Glu Ala Ala Gly Thr Tyr Val
260 265 270

Ala Ala Asp Ala Ala Ala Ala Ser Thr Tyr Thr Gly Phe Asp Ile Met
25 275 280 285

Asp Phe Gly Leu Leu Pro Pro Glu Val Asn Ser Ser Arg Met Tyr Ser
290 295 300

Gly Pro Gly Pro Glu Ser Met Leu Ala Ala Ala Ala Ala Trp Asp Gly
30 305 310 315 320

Val Ala Ala Glu Leu Thr Ser Ala Ala Val Ser Tyr Gly Ser Val Val
325 330 335

35 Ser Thr Leu Ile Val Glu Pro Trp Met Gly Pro Ala Ala Ala Ala Met
340 345 350

Ala Ala Ala Ala Thr Pro Tyr Val Gly Trp Leu Ala Ala Thr Ala Ala
355 360 365

40

Leu Ala Lys Glu Thr Ala Thr Gln Ala Arg Ala Ala Ala Glu Ala Phe
370 375 380

Gly Thr Ala Phe Ala Met Thr Val Pro Pro Ser Leu Val Ala Ala Asn
45 385 390 395 400

Arg Ser Arg Leu Met Ser Leu Val Ala Ala Asn Ile Leu Gly Gln Asn
405 410 415

50 Ser Ala Ala Ile Ala Ala Thr Gln Ala Glu Tyr Ala Glu Met Trp Ala
420 425 430

Gln Asp Ala Ala Val Met Tyr Ser Tyr Glu Gly Ala Ser Ala Ala Ala
435 440 445

55

Ser Ala Leu Pro Pro Phe Thr Pro Pro Val Gln Gly Thr Gly Pro Ala
450 455 460

Gly Pro Ala Ala Ala Ala Ala Ala Thr Gln Ala Ala Gly Ala Gly Ala
60 465 470 475 480

Val Ala Asp Ala Gln Ala Thr Leu Ala Gln Leu Pro Pro Gly Ile Leu
485 490 495

65 Ser Asp Ile Leu Ser Ala Leu Ala Ala Asn Ala Asp Pro Leu Thr Ser
500 505 510

Gly Leu Leu Gly Ile Ala Ser Thr Leu Asn Pro Gln Val Gly Ser Ala

```
        515                 520                 525

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

Leu Tyr Ile Ala Ser Ile Ala Thr Gly Ser Ile Ala Leu Ala Ile Thr
545                 550                 555                 560

Asn Thr Ala Arg Pro Trp His Ile Gly Leu Tyr Gly Asn Ala Gly Gly
                565                 570                 575

Leu Gly Pro Thr Gln Gly His Pro Leu Ser Ser Ala Thr Asp Glu Pro
            580                 585                 590

Glu Pro His Trp Gly Pro Phe Gly Gly Ala Ala Pro Val Ser Ala Gly
        595                 600                 605

Val Gly His Ala Ala Leu Val Gly Ala Leu Ser Val Pro His Ser Trp
    610                 615                 620

Thr Thr Ala Ala Pro Glu Ile Gln Leu Ala Val Gln Ala Thr Pro Thr
625                 630                 635                 640

Phe Ser Ser Ser Ala Gly Ala Asp Pro Thr Ala Leu Asn Gly Met Pro
                645                 650                 655

Ala Gly Leu Leu Ser Gly Met Ala Leu Ala Ser Leu Ala Ala Arg Gly
            660                 665                 670

Thr Thr Gly Gly Gly Gly Thr Arg Ser Gly Thr Ser Thr Asp Gly Gln
        675                 680                 685

Glu Asp Gly Arg Lys Pro Pro Val Val Val Ile Arg Glu Gln Pro Pro
    690                 695                 700

Pro Gly Asn Pro Pro Arg
705                 710


<211> 873
<212> DNA
<213> Leish-Tb (MAPS-DPVpET)
<400> SEQ ID NO:50

atgcatcacc atcaccatca catgtcctgc ggtaacgcca agatcaactc tcccgcgccg 60
tccttcgagg aggtggcgct catgcccaac ggcagcttca agaagatcag cctctcctcc 120
tacaagggca agtgggtcgt gctcttcttc tacccgctcg acttcacctt cgtgtgcccg 180
acagaggtca tcgcgttctc cgacagcgtg agtcgcttca acgagctcaa ctgcgaggtc 240
ctcgcgtgct cgatagacag cgagtacgcg cacctgcagt ggacgctgca ggaccgcaag 300
aagggcggcc tcgggaccat ggcgatccca atgctagccg acaagaccaa gagcatcgct 360
cgttcctacg gcgtgctgga ggagagccag ggcgtggcct accgcggtct cttcatcatc 420
gacccccatg gcatgctgcg tcagatcacc gtcaatgaca tgccggtggg ccgcagcgtg 480
gaggaggttc tacgcctgct ggaggctttt cagttcgtgg agaagcacgg cgaggtgtgc 540
cccgcgaact ggaagaaggg cgcccccacg atgaagccgg aaccgaatgc gtctgtcgag 600
ggatacttca gcaagcagga attcgatccc gtggacgcgg tcattaacac cacctgcaat 660
tacgggcagg tagtagctgc gctcaacgcg acggatccgg gggctgccgc acagttcaac 720
gcctcaccgg tggcgcagtc ctatttgcgc aatttcctcg ccgcaccgcc acctcagcgc 780
gctgccatgg ccgcgcaatt gcaagctgtg ccgggggcgg cacagtacat cggccttgtc 840
gagtcggttg ccggctcctg caacaactat taa                              873


<211> 290
<212> PRT
<213> Leish-Tb (MAPS-DPVpET protein)
<400> SEQ ID NO:51
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Met His His His His His His Met Ser Cys Gly Asn Ala Lys Ile Asn
                    5                   10                  15

Ser Pro Ala Pro Ser Phe Glu Glu Val Ala Leu Met Pro Asn Gly Ser
            20                  25                  30

Phe Lys Lys Ile Ser Leu Ser Ser Tyr Lys Gly Lys Trp Val Val Leu
        35                  40                  45

Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro Thr Glu Val Ile
    50                  55                  60

Ala Phe Ser Asp Ser Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val
65                  70                  75                  80

Leu Ala Cys Ser Ile Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu
                85                  90                  95

Gln Asp Arg Lys Lys Gly Gly Leu Gly Thr Met Ala Ile Pro Met Leu
            100                 105                 110

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

Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro His Gly
    130                 135                 140

Met Leu Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg Ser Val
145                 150                 155                 160

Glu Glu Val Leu Arg Leu Leu Glu Ala Phe Gln Phe Val Glu Lys His
                165                 170                 175

Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys
            180                 185                 190

Pro Glu Pro Asn Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln Glu Phe
        195                 200                 205

Asp Pro Val Asp Ala Val Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val
    210                 215                 220

Val Ala Ala Leu Asn Ala Thr Asp Pro Gly Ala Ala Ala Gln Phe Asn
225                 230                 235                 240

Ala Ser Pro Val Ala Gln Ser Tyr Leu Arg Asn Phe Leu Ala Ala Pro
                245                 250                 255

Pro Pro Gln Arg Ala Ala Met Ala Ala Gln Leu Gln Ala Val Pro Gly
            260                 265                 270

Ala Ala Gln Tyr Ile Gly Leu Val Glu Ser Val Ala Gly Ser Cys Asn
        275                 280                 285

Asn Tyr
    290
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<211> 987
<212> DNA

<213> Leish-Tb (MAPS-DPASpET)
<400> SEQ ID NO:52

atgcatcacc atcaccatca catgtcctgc ggtaacgcca agatcaactc tcccgcgccg 60
5 tccttcgagg aggtggcgct catgcccaac ggcagcttca agaagatcag cctctcctcc 120
tacaagggca agtgggtcgt gctcttcttc tacccgctcg acttcacctt cgtgtgcccg 180
acagaggtca tcgcgttctc cgacagcgtg agtcgcttca acgagctcaa ctgcgaggtc 240
ctcgcgtgct cgatagacag cgagtacgcg cacctgcagt ggacgctgca ggaccgcaag 300
aagggcggcc tcgggaccat ggcgatccca atgctagccg acaagaccaa gagcatcgct 360
10 cgttcctacg gcgtgctgga ggagagccag ggcgtggcct accgcggtct cttcatcatc 420
gaccccccatg gcatgctgcg tcagatcacc gtcaatgaca tgccggtggg ccgcagcgtg 480
gaggaggttc tacgcctgct ggaggctttt cagttcgtgg agaagcacgg cgaggtgtgc 540
cccgcgaact ggaagaaggg cgccccccacg atgaagccgg aaccgaatgc gtctgtcgag 600
ggatacttca gcaagcagga attcgacccg gcatccgccc ctgacgtccc gaccgccgcc 660
15 cagttgacca gcctgctcaa cagcctcgcc gatcccaacg tgtcgtttgc gaacaagggc 720
agtctggtcg agggcggcat cgggggcacc gaggcgcgca tcgccgacca caagctgaag 780
aaggccgccg agcacgggga tctgccgctg tcgttcagcg tgacgaacat ccagccggcg 840
gccgccggtt cggccaccgc cgacgtttcc gtctcgggtc cgaagctctc gtcgccggtc 900
acgcagaacg tcacgttcgt gaatcaaggc ggctggatgc tgtcacgcgc atcggcgatg 960
20 gagttgctgc aggccgcagg gaactaa 987

<211> 328
<212> PRT
25 <213> Leish-Tb (MAPS-DPASpET protein)
<400> SEQ ID NO:53

Met His His His His His His Met Ser Cys Gly Asn Ala Lys Ile Asn
5 10 15
30
Ser Pro Ala Pro Ser Phe Glu Glu Val Ala Leu Met Pro Asn Gly Ser
20 25 30

Phe Lys Lys Ile Ser Leu Ser Ser Tyr Lys Gly Lys Trp Val Val Leu
35 35 40 45

Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro Thr Glu Val Ile
50 55 60

40 Ala Phe Ser Asp Ser Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val
65 70 75 80

Leu Ala Cys Ser Ile Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu
85 90 95
45
Gln Asp Arg Lys Lys Gly Gly Leu Gly Thr Met Ala Ile Pro Met Leu
100 105 110

Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu Glu Glu
50 115 120 125

Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro His Gly
130 135 140

55 Met Leu Arg Gln Ile Thr Val Asn Asp Met Pro Val Gly Arg Ser Val
145 150 155 160

Glu Glu Val Leu Arg Leu Leu Glu Ala Phe Gln Phe Val Glu Lys His
165 170 175
60
Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys

180 185 190

Pro Glu Pro Asn Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln Glu Phe
195 200 205

Asp Pro Ala Ser Ala Pro Asp Val Pro Thr Ala Ala Gln Leu Thr Ser
210 215 220

Leu Leu Asn Ser Leu Ala Asp Pro Asn Val Ser Phe Ala Asn Lys Gly
225 230 235 240

Ser Leu Val Glu Gly Gly Ile Gly Gly Thr Glu Ala Arg Ile Ala Asp
245 250 255

His Lys Leu Lys Lys Ala Ala Glu His Gly Asp Leu Pro Leu Ser Phe
260 265 270

Ser Val Thr Asn Ile Gln Pro Ala Ala Ala Gly Ser Ala Thr Ala Asp
275 280 285

Val Ser Val Ser Gly Pro Lys Leu Ser Ser Pro Val Thr Gln Asn Val
290 295 300

Thr Phe Val Asn Gln Gly Gly Trp Met Leu Ser Arg Ala Ser Ala Met
305 310 315 320

Glu Leu Leu Gln Ala Ala Gly Asn
325

<211> 852
<212> DNA
<213> Leish-Tb (MAPS-DPVpcDNA)
<400> SEQ ID NO:54

atgtcctgcg gtaacgccaa gatcaactct cccgcgccgt ccttcgagga ggtggcgctc 60
atgcccaacg gcagcttcaa gaagatcagc ctctcctcct acaagggcaa gtgggtcgtg 120
ctcttcttct acccgctcga cttcaccttc gtgtgcccga cagaggtcat cgcgttctcc 180
gacagcgtga gtcgcttcaa cgagctcaac tgcgaggtcc tcgcgtgctc gatagacagc 240
gagtacgcgc acctgcagtg gacgctgcag gaccgcaaga agggcggcct cgggaccatg 300
gcgatcccaa tgctagccga caagaccaag agcatcgctc gttcctacgg cgtgctggag 360
gagagccagg gcgtggccta ccgcggtctc ttcatcatcg acccccatgg catgctgcgt 420
cagatcaccg tcaatgacat gccggtgggc cgcagcgtgg aggaggttct acgcctgctg 480
gaggcttttc agttcgtgga gaagcacggc gaggtgtgcc ccgcgaactg gaagaagggc 540
gcccccacga tgaagccgga accgaatgcg tctgtcgagg gatacttcag caagcaggaa 600
ttcgatcccg tggacgcggt cattaacacc acctgcaatt acgggcaggt agtagctgcg 660
ctcaacgcga cggatccggg ggctgccgca cagttcaacg cctcaccggt ggcgcagtcc 720
tatttgcgca atttcctcgc cgcaccgcca cctcagcgcg ctgccatggc cgcgcaattg 780
caagctgtgc cgggggcggc acagtacatc ggccttgtcg agtcggttgc cggctcctgc 840
aacaactatt aa 852

<211> 966
<212> DNA
<213> Leish-Tb (MAPS-DPVpcDNA)
<400> SEQ ID NO:55

atgtcctgcg gtaacgccaa gatcaactct cccgcgccgt ccttcgagga ggtggcgctc 60
atgcccaacg gcagcttcaa gaagatcagc ctctcctcct acaagggcaa gtgggtcgtg 120
ctcttcttct acccgctcga cttcaccttc gtgtgcccga cagaggtcat cgcgttctcc 180
gacagcgtga gtcgcttcaa cgagctcaac tgcgaggtcc tcgcgtgctc gatagacagc 240

gagtacgcgc acctgcagtg gacgctgcag gaccgcaaga agggcggcct cgggaccatg 300
gcgatcccaa tgctagccga caagaccaag agcatcgctc gttcctacgg cgtgctggag 360
gagagccagg gcgtggccta ccgcggtctc ttcatcatcg acccccatgg catgctgcgt 420
cagatcaccg tcaatgacat gccggtgggc cgcagcgtgg aggaggttct acgcctgctg 480
5 gaggcttttc agttcgtgga gaagcacggc gaggtgtgcc ccgcgaactg gaagaagggc 540
gcccccacga tgaagccgga accgaatgcg tctgtcgagg gatacttcag caagcaggaa 600
ttcgacccgg catccgcccc tgacgtcccg accgccgccc agttgaccag cctgctcaac 660
agcctcgccg atcccaacgt gtcgtttgcg aacaagggca gtctggtcga gggcggcatc 720
gggggcaccg aggcgcgcat cgccgaccac aagctgaaga aggccgccga gcacggggat 780
10 ctgccgctgt cgttcagcgt gacgaacatc cagccggcgg ccgccggttc ggccaccgcc 840
gacgtttccg tctcgggtcc gaagctctcg tcgccggtca cgcagaacgt cacgttcgtg 900
aatcaaggcg gctggatgct gtcacgcgca tcggcgatgg agttgctgca ggccgcaggg 960
aactaa 966

15

<211> 864
<212> DNA
<213> *Leishmania* major / *M. tuberculosis* (MAPS-DPV-AC)
<400> SEQ ID NO:56

20

ggatccatgt cctgcggtaa cgccaagatc aactctcccg cgccgtcctt cgaggaggtg 60
gcgctcatgc ccaacggcag cttcaagaag atcagcctct cctcctacaa gggcaagtgg 120
gtcgtgctct tcttctaccc gctcgacttc accttcgtgt gcccgacaga ggtcatcgcg 180
ttctccgaca gcgtgagtcg cttcaacgag ctcaactgcg aggtcctcgc gtgctcgata 240
25 gacagcgagt acgcgcacct gcagtggacg ctgcaggacc gcaagaaggg cggcctcggg 300
accatggcga tcccaatgct agccgacaag accaagagca tcgctcgttc ctacggcgtg 360
ctggaggaga gccagggcgt ggcctaccgc ggtctcttca tcatcgaccc ccatggcatg 420
ctgcgtcaga tcaccgtcaa tgacatgccg gtgggccgca gcgtggagga ggttctacgc 480
ctgctggagg cttttcagtt cgtggagaag cacggcgagg tgtgccccgc gaactggaag 540
30 aagggcgccc ccacgatgaa gccggaaccg aatgcgtctg tcgagggata cttcagcaag 600
caggaattcg accccgtgga cgccgtgatc aacaccacct gcaactacgg ccaggtggtg 660
gctgccctga acgcgaccga ccccggcgct gccgcacagt tcaacgcctc ccctgtggcc 720
cagtcctacc tgcgcaactt cctcgccgca cccccacctc agcgcgctgc catggccgcc 780
cagctgcagg ctgtgcccgg cgccgcacag tacatcggcc tggtcgagtc cgtggccggc 840
35 tcctgcaaca actactaaga attc 864

<211> 284
<212> PRT
40 <213> *Leishmania* major / *M. tuberculosis* (MAPS-DPV-AC protein)
<400> SEQ ID NO:57

Met Ser Cys Gly Asn Ala Lys Ile Asn Ser Pro Ala Pro Ser Phe Glu
5 10 15

45

Glu Val Ala Leu Met Pro Asn Gly Ser Phe Lys Lys Ile Ser Leu Ser
20 25 30

Ser Tyr Lys Gly Lys Trp Val Val Leu Phe Phe Tyr Pro Leu Asp Phe
50 35 40 45

Thr Phe Val Cys Pro Thr Glu Val Ile Ala Phe Ser Asp Ser Val Ser
50 55 60

55 Arg Phe Asn Glu Leu Asn Cys Glu Val Leu Ala Cys Ser Ile Asp Ser
65 70 75 80

Glu Tyr Ala His Leu Gln Trp Thr Leu Gln Asp Arg Lys Lys Gly Gly
85 90 95

60

Leu Gly Thr Met Ala Ile Pro Met Leu Ala Asp Lys Thr Lys Ser Ile

100 105 110

Ala Arg Ser Tyr Gly Val Leu Glu Glu Ser Gln Gly Val Ala Tyr Arg
115 120 125

Gly Leu Phe Ile Ile Asp Pro His Gly Met Leu Arg Gln Ile Thr Val
130 135 140

Asn Asp Met Pro Val Gly Arg Ser Val Glu Glu Val Leu Arg Leu Leu
145 150 155 160

Glu Ala Phe Gln Phe Val Glu Lys His Gly Glu Val Cys Pro Ala Asn
165 170 175

Trp Lys Lys Gly Ala Pro Thr Met Lys Pro Glu Pro Asn Ala Ser Val
180 185 190

Glu Gly Tyr Phe Ser Lys Gln Glu Phe Asp Pro Val Asp Ala Val Ile
195 200 205

Asn Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr
210 215 220

Asp Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser
225 230 235 240

Tyr Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met
245 250 255

Ala Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu
260 265 270

Val Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr
275 280

<211> 978
<212> DNA
<213> *Leishmania* major / *M. tuberculosis* (MAPS-DPAS-AC)
<400> SEQ ID NO:58

ggatccatgt cctgcggtaa cgccaagatc aactctcccg cgccgtcctt cgaggaggtg 60
gcgctcatgc ccaacggcag cttcaagaag atcagcctct cctcctacaa gggcaagtgg 120
gtcgtgctct tcttctaccc gctcgacttc accttcgtgt gcccgacaga ggtcatcgcg 180
ttctccgaca gcgtgagtcg cttcaacgag ctcaactgcg aggtcctcgc gtgctcgata 240
gacagcgagt acgcgcacct gcagtggacg ctgcaggacc gcaagaaggg cggcctcggg 300
accatggcga tcccaatgct agccgacaag accaagagca tcgctcgttc ctacggcgtg 360
ctggaggaga gccagggcgt ggcctaccgc ggtctcttca tcatcgaccc ccatggcatg 420
ctgcgtcaga tcaccgtcaa tgacatgccg gtgggccgca gcgtggagga ggttctacgc 480
ctgctggagg cttttcagtt cgtggagaag cacggcgagg tgtgccccgc gaactggaag 540
aagggcgccc ccacgatgaa gccggaaccg aatgcgtctg tcgagggata cttcagcaag 600
caggaattcg accccgcctc cgcccctgac gtgcccaccg ccgcccagct gaccagcctg 660
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<211> 322

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Ser Tyr Lys Gly Lys Trp Val Val Leu Phe Phe Tyr Pro Leu Asp Phe
35 40 45

Thr Phe Val Cys Pro Thr Glu Val Ile Ala Phe Ser Asp Ser Val Ser
50 55 60

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

Glu Tyr Ala His Leu Gln Trp Thr Leu Gln Asp Arg Lys Lys Gly Gly
85 90 95

Leu Gly Thr Met Ala Ile Pro Met Leu Ala Asp Lys Thr Lys Ser Ile
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Ala Arg Ser Tyr Gly Val Leu Glu Glu Ser Gln Gly Val Ala Tyr Arg
115 120 125

Gly Leu Phe Ile Ile Asp Pro His Gly Met Leu Arg Gln Ile Thr Val
130 135 140

Asn Asp Met Pro Val Gly Arg Ser Val Glu Glu Val Leu Arg Leu Leu
145 150 155 160

Glu Ala Phe Gln Phe Val Glu Lys His Gly Glu Val Cys Pro Ala Asn
165 170 175

Trp Lys Lys Gly Ala Pro Thr Met Lys Pro Glu Pro Asn Ala Ser Val
180 185 190

Glu Gly Tyr Phe Ser Lys Gln Glu Phe Asp Pro Ala Ser Ala Pro Asp
195 200 205

Val Pro Thr Ala Ala Gln Leu Thr Ser Leu Leu Asn Ser Leu Ala Asp
210 215 220

Pro Asn Val Ser Phe Ala Asn Lys Gly Ser Leu Val Glu Gly Gly Ile
225 230 235 240

Gly Gly Thr Glu Ala Arg Ile Ala Asp His Lys Leu Lys Lys Ala Ala
245 250 255

Glu His Gly Asp Leu Pro Leu Ser Phe Ser Val Thr Asn Ile Gln Pro
260 265 270

Ala Ala Ala Gly Ser Ala Thr Ala Asp Val Ser Val Ser Gly Pro Lys
275 280 285

Leu Ser Ser Pro Val Thr Gln Asn Val Thr Phe Val Asn Gln Gly Gly
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Trp Met Leu Ser Arg Ala Ser Ala Met Glu Leu Leu Gln Ala Ala Gly
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Asn

<212> DNA
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gccggctcct gcaacaacta ctaa 264

<211> 88
<212> PRT
<213> *M. tuberculosis* (MAPS(N5)-DPV-AC protein)
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Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala Thr Asp Pro Gly Ala
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Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln Ser Tyr Leu Arg Asn
35 40 45

Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala Met Ala Ala Gln Leu
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Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly Leu Val Glu Ser Val
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Ala Gly Ser Cys Asn Asn Tyr
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<211> 93
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Met Ser Cys Gly Asn Ala Lys Ile Asn Ser Asp Pro Val Asp Ala Val
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Ile Asn Thr Thr Cys Asn Tyr Gly Gln Val Val Ala Ala Leu Asn Ala
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5 Thr Asp Pro Gly Ala Ala Ala Gln Phe Asn Ala Ser Pro Val Ala Gln
35 40 45

Ser Tyr Leu Arg Asn Phe Leu Ala Ala Pro Pro Pro Gln Arg Ala Ala
50 55 60

10

Met Ala Ala Gln Leu Gln Ala Val Pro Gly Ala Ala Gln Tyr Ile Gly
65 70 75 80

Leu Val Glu Ser Val Ala Gly Ser Cys Asn Asn Tyr
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20 <213> *M. tuberculosis* (MTB72F-MAPS)
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Ile Ala Gly Gln Ile Arg Ser Gly Gly Gly Ser Pro Thr Val His Ile
35 40 45

Gly Pro Thr Ala Phe Leu Gly Leu Gly Val Val Asp Asn Asn Gly Asn
50 55 60

Gly Ala Arg Val Gln Arg Val Val Gly Ser Ala Pro Ala Ala Ser Leu
65 70 75 80

Gly Ile Ser Thr Gly Asp Val Ile Thr Ala Val Asp Gly Ala Pro Ile
85 90 95

Asn Ser Ala Thr Ala Met Ala Asp Ala Leu Asn Gly His His Pro Gly
100 105 110

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

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

Phe Gly Ala Leu Pro Pro Glu Ile Asn Ser Ala Arg Met Tyr Ala Gly
145 150 155 160

Pro Gly Ser Ala Ser Leu Val Ala Ala Ala Gln Met Trp Asp Ser Val
165 170 175

Ala Ser Asp Leu Phe Ser Ala Ala Ser Ala Phe Gln Ser Val Val Trp
180 185 190

Gly Leu Thr Val Gly Ser Trp Ile Gly Ser Ser Ala Gly Leu Met Val
195 200 205

Ala Ala Ala Ser Pro Tyr Val Ala Trp Met Ser Val Thr Ala Gly Gln
210 215 220

Ala Glu Leu Thr Ala Ala Gln Val Arg Val Ala Ala Ala Ala Tyr Glu
225 230 235 240

Thr Ala Tyr Gly Leu Thr Val Pro Pro Pro Val Ile Ala Glu Asn Arg

245 250 255

Ala Glu Leu Met Ile Leu Ile Ala Thr Asn Leu Leu Gly Gln Asn Thr
260 265 270

5

Pro Ala Ile Ala Val Asn Glu Ala Glu Tyr Gly Glu Met Trp Ala Gln
275 280 285

Asp Ala Ala Ala Met Phe Gly Tyr Ala Ala Ala Thr Ala Thr Ala Thr
10 290 295 300

Ala Thr Leu Leu Pro Phe Glu Glu Ala Pro Glu Met Thr Ser Ala Gly
305 310 315 320

15 Gly Leu Leu Glu Gln Ala Ala Ala Val Glu Glu Ala Ser Asp Thr Ala
325 330 335

Ala Ala Asn Gln Leu Met Asn Asn Val Pro Gln Ala Leu Gln Gln Leu
340 345 350

20

Ala Gln Pro Thr Gln Gly Thr Thr Pro Ser Ser Lys Leu Gly Gly Leu
355 360 365

Trp Lys Thr Val Ser Pro His Arg Ser Pro Ile Ser Asn Met Val Ser
25 370 375 380

Met Ala Asn Asn His Met Ser Met Thr Asn Ser Gly Val Ser Met Thr
385 390 395 400

30 Asn Thr Leu Ser Ser Met Leu Lys Gly Phe Ala Pro Ala Ala Ala Ala
405 410 415

Gln Ala Val Gln Thr Ala Ala Gln Asn Gly Val Arg Ala Met Ser Ser
420 425 430

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Leu Gly Ser Ser Leu Gly Ser Ser Gly Leu Gly Gly Gly Val Ala Ala
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Asn Leu Gly Arg Ala Ala Ser Val Gly Ser Leu Ser Val Pro Gln Ala
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Trp Ala Ala Ala Asn Gln Ala Val Thr Pro Ala Ala Arg Ala Leu Pro
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45 Leu Thr Ser Leu Thr Ser Ala Ala Glu Arg Gly Pro Gly Gln Met Leu
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Gly Gly Leu Pro Val Gly Gln Met Gly Ala Arg Ala Gly Gly Gly Leu
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50

Ser Gly Val Leu Arg Val Pro Pro Arg Pro Tyr Val Met Pro His Ser
515 520 525

Pro Ala Ala Gly Asp Ile Ala Pro Pro Ala Leu Ser Gln Asp Arg Phe
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Ala Asp Phe Pro Ala Leu Pro Leu Asp Pro Ser Ala Met Val Ala Gln
545 550 555 560

60 Val Gly Pro Gln Val Val Asn Ile Asn Thr Lys Leu Gly Tyr Asn Asn
565 570 575

Ala Val Gly Ala Gly Thr Gly Ile Val Ile Asp Pro Asn Gly Val Val
580 585 590

Leu Thr Asn Asn His Val Ile Ala Gly Ala Thr Asp Ile Asn Ala Phe
595 600 605

Ser Val Gly Ser Gly Gln Thr Tyr Gly Val Asp Val Val Gly Tyr Asp
610 615 620

Arg Thr Gln Asp Val Ala Val Leu Gln Leu Arg Gly Ala Gly Gly Leu
625 630 635 640

Pro Ser Ala Ala Ile Gly Gly Gly Val Ala Val Gly Glu Pro Val Val
645 650 655

Ala Met Gly Asn Ser Gly Gly Gln Gly Gly Thr Pro Arg Ala Val Pro
660 665 670

Gly Arg Val Val Ala Leu Gly Gln Thr Val Gln Ala Ser Asp Ser Leu
675 680 685

Thr Gly Ala Glu Glu Thr Leu Asn Gly Leu Ile Gln Phe Asp Ala Ala
690 695 700

Ile Gln Pro Gly Asp Ser Gly Gly Pro Val Val Asn Gly Leu Gly Gln
705 710 715 720

Val Val Gly Met Asn Thr Ala Ala Ser Gly Thr Met Ser Cys Gly Asn
725 730 735

Ala Lys Ile Asn Ser Pro Ala Pro Ser Phe Glu Glu Val Ala Leu Met
740 745 750

Pro Asn Gly Ser Phe Lys Lys Ile Ser Leu Ser Ser Tyr Lys Gly Lys
755 760 765

Trp Val Val Leu Phe Phe Tyr Pro Leu Asp Phe Thr Phe Val Cys Pro
770 775 780

Thr Glu Val Ile Ala Phe Ser Asp Ser Val Ser Arg Phe Asn Glu Leu
785 790 795 800

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Gln Trp Thr Leu Gln Asp Arg Lys Lys Gly Gly Leu Gly Thr Met Ala
820 825 830

Ile Pro Met Leu Ala Asp Lys Thr Lys Ser Ile Ala Arg Ser Tyr Gly
835 840 845

Val Leu Glu Glu Ser Gln Gly Val Ala Tyr Arg Gly Leu Phe Ile Ile
850 855 860

Asp Pro His Gly Met Leu Arg Gln Ile Thr Val Asn Asp Met Pro Val
865 870 875 880

Gly Arg Ser Val Glu Glu Val Leu Arg Leu Leu Glu Ala Phe Gln Phe
885 890 895

Val Glu Lys His Gly Glu Val Cys Pro Ala Asn Trp Lys Lys Gly Ala
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Pro Thr Met Lys Pro Glu Pro Asn Ala Ser Val Glu Gly Tyr Phe Ser
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Lys Gln
930

10

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gaacgtgaca ttcgtgaacc agggcggctg gatgctgtcc cgcgcctccg ctatggagct 360
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<211> 849
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Pro Pro Pro Gln Arg Ala Ala Met Ala Ala Gln Leu Gln Ala Val Pro
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Gly Ala Ala Gln Tyr Ile Gly Leu Val Glu Ser Val Ala Gly Ser Cys
65                  70                  75                  80

Asn Asn Tyr Met Ser Cys Gly Asn Ala Lys Ile Asn Ser Pro Ala Pro
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Ser Phe Glu Glu Val Ala Leu Met Pro Asn Gly Ser Phe Lys Lys Ile
            100                 105                 110

Ser Leu Ser Ser Tyr Lys Gly Lys Trp Val Val Leu Phe Phe Tyr Pro
        115                 120                 125

Leu Asp Phe Thr Phe Val Cys Pro Thr Glu Val Ile Ala Phe Ser Asp
    130                 135                 140

Ser Val Ser Arg Phe Asn Glu Leu Asn Cys Glu Val Leu Ala Cys Ser
145                 150                 155                 160

Ile Asp Ser Glu Tyr Ala His Leu Gln Trp Thr Leu Gln Asp Arg Lys
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Lys Gly Gly Leu Gly Thr Met Ala Ile Pro Met Leu Ala Asp Lys Thr
            180                 185                 190

Lys Ser Ile Ala Arg Ser Tyr Gly Val Leu Glu Glu Ser Gln Gly Val
        195                 200                 205

Ala Tyr Arg Gly Leu Phe Ile Ile Asp Pro His Gly Met Leu Arg Gln
    210                 215                 220

Ile Thr Val Asn Asp Met Pro Val Gly Arg Ser Val Glu Glu Val Leu
225                 230                 235                 240

Arg Leu Leu Glu Ala Phe Gln Phe Val Glu Lys His Gly Glu Val Cys
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Pro Ala Asn Trp Lys Lys Gly Ala Pro Thr Met Lys Pro Glu Pro Asn
            260                 265                 270

Ala Ser Val Glu Gly Tyr Phe Ser Lys Gln
        275                 280
```

WHAT IS CLAIMED IS:

1 1. An expression cassette comprising a recombinant nucleic acid
2 molecule encoding a fusion polypeptide, the recombinant nucleic acid molecule comprising a
3 heterologous polynucleotide sequence encoding an antigen or an antigenic fragment, and a
4 *Leishmania* polynucleotide sequence encoding a polypeptide or a fragment thereof, wherein
5 the *Leishmania* polynucleotide is selected from the group consisting of TSA polynucleotide,
6 LeIF polynucleotide, M15 polynucleotide, and 6H polynucleotide, and wherein the
7 recombinant nucleic acid molecule is operably linked to a eukaryotic promoter.

1 2. The expression cassette of claim 1, wherein the *Leishmania*
2 polynucleotide sequence is the TSA polynucleotide.

1 3. The expression cassette of claim 1, wherein the *Leishmania*
2 polynucleotide sequence is the TSA polynucleotide.

1 4. The expression cassette of claim 2, wherein the TSA polynucleotide is
2 a N-terminal fragment of the *Leishmania* thiol-specific-antioxidant gene.

1 5. The expression cassette of claim 4, wherein the N-terminal fragment of
2 the *Leishmania* thiol-specific-antioxidant gene comprises about 30 or less nucleotides.

1 6. The expression cassette of claim 1, wherein the heterologous
2 polynucleotide sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a
3 bacterial antigen.

1 7. The expression cassette of claim 6, wherein the heterologous
2 polynucleotide sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or
3 antigenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 8. The expression cassette of claim 7, wherein the *Mycobacterium* species
2 is *Mycobacterium tuberculosis*.

1 9. The expression cassette of claim 7, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen,
3 MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35
4 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4

5 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex
6 antigen, or an immunogenic fragment thereof.

1 10. The expression cassette of claim 7, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen,
3 MTB71F antigen, MTB103F, or an immunogenic fragment thereof.

1 11. The expression cassette of claim 2, wherein the heterologous
2 polynucleotide sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 12. The expression cassette of claim 1, wherein the *Leishmania*
2 polynucleotide sequence is located 5' to the heterologous polynucleotide sequence.

1 13. The expression cassette of claim 1, wherein the *Leishmania*
2 polynucleotide sequence is located 3' to the heterologous polynucleotide sequence.

1 14. The expression cassette of claim 1, wherein the heterologous
2 polynucleotide sequence is codon optimized for expression in eukaryotic cells.

1 15. The expression cassette of claim 14, wherein the *Leishmania*
2 polynucleotide sequence is the TSA polynucleotide.

1 16. The expression cassette of claim 14, wherein the heterologous
2 polynucleotide sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a
3 bacterial antigen.

1 17. The expression cassette of claim 16, wherein the heterologous
2 polynucleotide sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or
3 antigenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 18. The expression cassette of claim 17, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen,
3 MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35
4 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4
5 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex
6 antigen, or an immunogenic fragment thereof.

1 19. The expression cassette of claim 17, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen,
3 MTB71F antigen, an MTB103F antigen or an immunogenic fragment thereof.

1 20. The expression cassette of claim 15, wherein the heterologous
2 polynucleotide sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 21. The expression cassette of claim 14, wherein the *Leishmania*
2 polynucleotide sequence is located 5' to the heterologous polynucleotide sequence.

1 22. The expression cassette of claim 14, wherein the *Leishmania*
2 polynucleotide sequence is located 3' to the heterologous polynucleotide sequence.

1 23. A host cell comprising the expression cassette of claim 1.

1 24. The host cell of claim 23, wherein the host cell is a eukaryotic cell.

1 25. A composition comprising an expression cassette comprising a
2 recombinant nucleic acid molecule encoding a fusion polypeptide, the recombinant nucleic
3 acid molecule comprising a heterologous polynucleotide sequence encoding an antigen or an
4 antigenic fragment, and a *Leishmania* polynucleotide sequence encoding a polypeptide or a
5 fragment thereof, wherein the *Leishmania* polynucleotide is selected from the group
6 consisting of TSA polynucleotide, LeIF polynucleotide, M15 polynucleotide, and 6H
7 polynucleotide, and wherein the recombinant nucleic acid molecule is operably linked to a
8 eukaryotic promoter.

1 26. The composition of claim 25, wherein the *Leishmania* polynucleotide
2 sequence is the TSA polynucleotide.

1 27. The composition of claim 25, wherein the heterologous polynucleotide
2 sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a bacterial antigen.

1 28. The composition of claim 27, wherein the heterologous polynucleotide
2 sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or antigenic
3 fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 29. The composition of claim 28, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen,
3 MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35
4 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4
5 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex
6 antigen, or an immunogenic fragment thereof.

1 30. The composition of claim 28, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen,
3 MTB71F antigen, MTB103F antigen, or an immunogenic fragment thereof.

1 31. The composition of claim 26, wherein the heterologous polynucleotide
2 sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 32. The composition of claim 25, wherein the *Mycobacterium*
2 polynucleotide sequence is codon optimized for expression in eukaryotic cells.

1 33. The composition of claim 32, wherein the *Leishmania* polynucleotide
2 sequence is TSA polynucleotide, LeIF polynucleotide, M15 polynucleotide, or 6H
3 polynucleotide.

1 34. The composition of claim 33, wherein the *Leishmania* polynucleotide
2 sequence is the TSA polynucleotide.

1 35. The composition of claim 32, wherein the heterologous polynucleotide
2 sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a bacterial antigen.

1 36. The composition of claim 35, wherein the heterologous polynucleotide
2 sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or antigenic
3 fragment thereof from a *Mycobacterium* species of tuberculosis complex.

1 37. The composition of claim 36, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen,
3 MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35
4 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4

5 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex
6 antigen, or an immunogenic fragment thereof.

1 38. The composition of claim 36, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen,
3 MTB71F antigen, an MTB103F antigen, or an immunogenic fragment thereof.

1 39. The composition of claim 34, wherein the heterologous polynucleotide
2 sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 40. The composition of claim 25, wherein the composition is effective
2 against infections by multiple microorganisms.

1 41. The composition of claim 40, wherein the multiple microorganisms are
2 *Leishmania* and *Mycobacterium*.

1 42. A method for eliciting an immune response in a mammal, the method
2 comprising the step of administering to the mammal an immunologically effective amount of
3 an expression cassette comprising a recombinant nucleic acid molecule encoding a fusion
4 polypeptide, the recombinant nucleic acid molecule comprising a heterologous
5 polynucleotide sequence encoding an antigen or an antigenic fragment, and a *Leishmania*
6 polynucleotide sequence encoding a polypeptide or a fragment thereof, wherein the
7 *Leishmania* polynucleotide is selected from the group consisting of TSA polynucleotide,
8 LeIF polynucleotide, M15 polynucleotide, and 6H polynucleotide, and wherein the
9 recombinant nucleic acid is operably linked to a eukaryotic promoter.

1 43. The method of claim 42, wherein the mammal is immunized with
2 BCG.

1 44. The method of claim 42, wherein the mammal is a human.

1 45. The method of claim 42, wherein the expression cassette is formulated
2 with an adjuvant.

1 46. The method of claim 45, wherein the adjuvant comprises QS21 and
2 MPL.

1 47. The method of claim 45, wherein the adjuvant is selected from the
2 group consisting of AS2, ENHANZYN, MPL, 3D-MPL, IFA, QS21, CWS, TDM, AGP,
3 CPG, Leif, saponin, saponin mimetics, or a combination thereof.

1 48. The method of claim 42, wherein the *Leishmania* polynucleotide
2 sequence is the TSA polynucleotide.

1 49. The method of claim 42, wherein the heterologous polynucleotide
2 sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a bacterial antigen.

1 50. The method of claim 49, wherein the heterologous polynucleotide
2 sequence is a *Mycobacterium* polynucleotide encoding an antigen or antigenic fragment
3 thereof from a *Mycobacterium* species of the tuberculosis complex.

1 51. The method of claim 50, wherein the *Mycobacterium* polynucleotide
2 sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB12 antigen,
3 MTB32A ANTIGEN, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35 antigen, Ra12
4 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4 antigen, DPPD
5 antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex antigen, or an
6 immunogenic fragment thereof.

1 52. The method of claim 50, wherein the *Mycobacterium* polynucleotide
2 sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen, MTB71F
3 antigen, MTB103F antigen, or an immunogenic fragment thereof.

1 53. The method of claim 48, wherein the heterologous polynucleotide
2 sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 54. The method of claim 42, wherein the *Mycobacterium* polynucleotide
2 sequence is codon optimized for expression in eukaryotic cells.

1 55. The method of claim 54, wherein the *Leishmania* polynucleotide
2 sequence is the TSA polynucleotide.

1 56. The method of claim 42, wherein the heterologous polynucleotide
2 sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a bacterial antigen.

1 57. The method of claim 56, wherein the heterologous polynucleotide
2 sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or antigenic
3 fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 58. The method of claim 57, wherein the *Mycobacterium* polynucleotide
2 sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB12 antigen,
3 MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35 antigen, Ra12
4 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4 antigen, DPPD
5 antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex antigen, or an
6 immunogenic fragment thereof.

1 59. The method of claim 57, wherein the *Mycobacterium* polynucleotide
2 sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen, MTB71F
3 antigen, an MTB103F antigen, or an immunogenic fragment thereof.

1 60. The method of claim 55, wherein the heterologous polynucleotide
2 sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 61. The method of claim 42, wherein the expression cassette is effective
2 against infections by multiple microorganisms.

1 62. The method of claim 61, wherein the microorganisms are *Leishmania*
2 and *Mycobacterium*.

1 63. A recombinant nucleic acid molecule encoding a fusion polypeptide,
2 the recombinant nucleic acid comprising a heterologous polynucleotide sequence encoding an
3 antigen or an antigenic fragment, and a *Leishmania* polynucleotide encoding a polypeptide or
4 a fragment thereof, wherein the *Leishmania* polynucleotide is selected from the group
5 consisting of TSA polynucleotide, LeIF polynucleotide, M15 polynucleotide, and 6H
6 polynucleotide.

1 64. The recombinant nucleic acid of claim 63, wherein the *Leishmania*
2 polynucleotide sequence is the TSA polynucleotide.

1 65. The recombinant nucleic acid of claim 63, wherein the heterologous
2 polynucleotide sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a
3 bacterial antigen.

1 66. The recombinant nucleic acid of claim 65, wherein the heterologous
2 polynucleotide sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or
3 antigenic fragment thereof from a *Mycobacterium* species of tuberculosis complex.

1 67. The recombinant nucleic acid of claim 66, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen,
3 MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35
4 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4
5 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex
6 antigen, or an immunogenic fragment thereof.

1 68. The recombinant nucleic acid of claim 66, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen,
3 MTB71F antigen, MTB103F antigen, or an immunogenic fragment thereof.

1 69. The recombinant nucleic acid of claim 64, wherein the heterologous
2 polynucleotide sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 70. The recombinant nucleic acid of claim 63, wherein the *Mycobacterium*
2 polynucleotide sequence is codon optimized for expression in eukaryotic cells.

1 71. The recombinant nucleic acid of claim 70, wherein the *Leishmania*
2 polynucleotide sequence is the TSA polynucleotide.

1 72. The recombinant nucleic acid of claim 70, wherein the heterologous
2 polynucleotide sequence encodes a malaria antigen, a cancer antigen, a viral antigen or a
3 bacterial antigen.

1 73. The recombinant nucleic acid of claim 72, wherein the heterologous
2 polynucleotide sequence is a *Mycobacterium* polynucleotide sequence encoding an antigen or
3 antigenic fragment thereof from a *Mycobacterium* species of the tuberculosis complex.

1 74. The recombinant nucleic acid of claim 73, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen,
3 MTB12 antigen, MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35
4 antigen, Ra12 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4
5 antigen, DPPD antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex
6 antigen, or an immunogenic fragment thereof.

1 75. The recombinant nucleic acid of claim 73, wherein the *Mycobacterium*
2 polynucleotide sequence encodes for MTB59F antigen, MTB72F antigen, MTB31F antigen,
3 MTB71F antigen, MTB103F antigen, or an immunogenic fragment thereof.

1 76. The recombinant nucleic acid of claim 71, wherein the heterologous
2 polynucleotide sequence encodes for MTB8.4 antigen or MTB12 antigen.

1 77. A fusion polypeptide comprising a heterologous antigen or an
2 antigenic thereof, and a *Leishmania* polypeptide or a fragment thereof, wherein the
3 *Leishmania* polypeptide is TSA, LeIF, M15, or 6H.

1 78. The fusion polypeptide of claim 77, wherein the *Leishmania*
2 polypeptide is TSA.

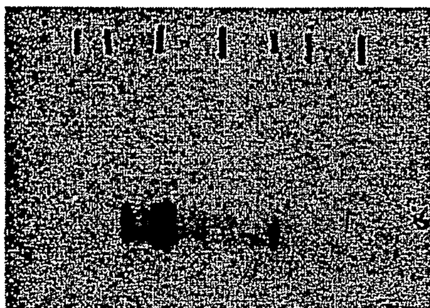
1 79. The fusion polypeptide of claim 77, wherein the heterologous antigen
2 is a malaria antigen, a cancer antigen, a viral antigen, or a bacterial antigen.

1 80. The fusion polypeptide of claim 79, wherein the heterologous antigen
2 is from a *Mycobacterium* species of the tuberculosis complex.

1 81. The fusion polypeptide of claim 80, wherein the *Mycobacterium*
2 polypeptide is MTB8.4 antigen, MTB9.8 antigen, MTB9.9 antigen, MTB12 antigen,
3 MTB32A antigen, MTB40 antigen, MTB41 antigen, TbH9 antigen, Ra35 antigen, Ra12
4 antigen, 38-1 antigen, TbRa3 antigen, 38 kD antigen, DPEP antigen, TbH4 antigen, DPPD
5 antigen, MTB82 antigen, Erd14 antigen, ESAT-6 antigen, MTB85b complex antigen, or an
6 immunogenic fragment thereof.

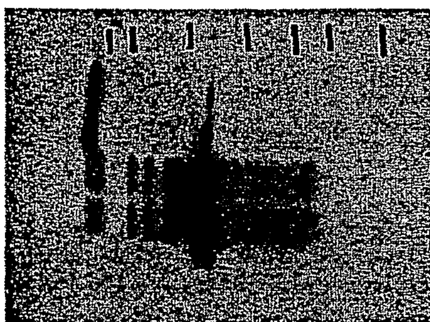
1 82. The fusion polypeptide of claim 80, wherein the *Mycobacterium*
2 polypeptide is MTB59F antigen, MTB72F antigen, MTB31F antigen, MTB71F antigen,
3 MTB103F antigen, or an immunogenic fragment thereof.

A



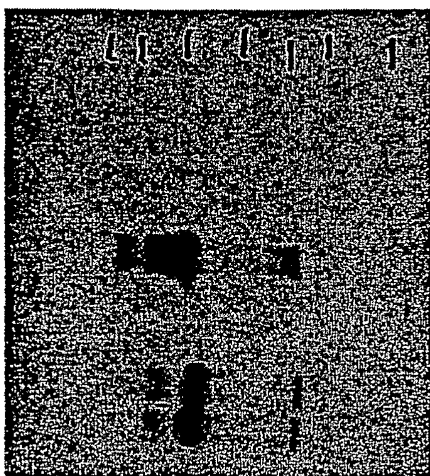
MWM
DPV
DPV-AC
MAPS-DPV
MAPS-DPV-AC
JA4304

B



MWM
DPAS
DPAS-AC
MAPS-DPAS
MAPS-DPAS-AC
JA4304

C



MWM
DPV
DPV-AC
MAPS-DPV
MAPS-DPV-AC
DPAS
DPAS-AC
MAPS-DPAS
MAPS-DPAS-AC
JA4304

Fig. 1

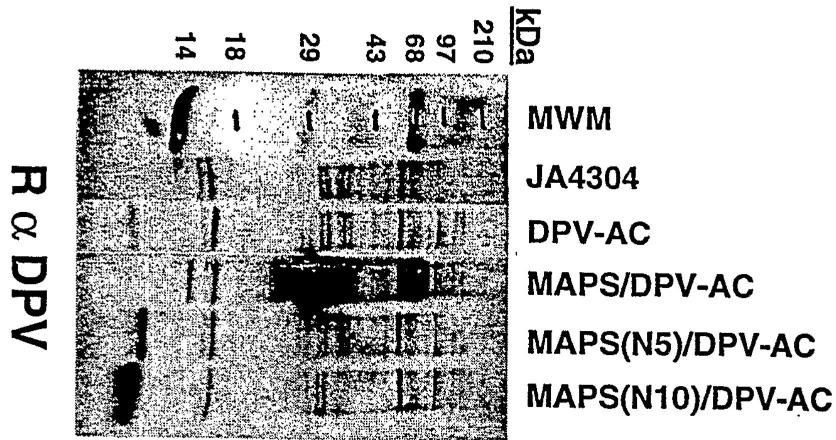


Fig. 2

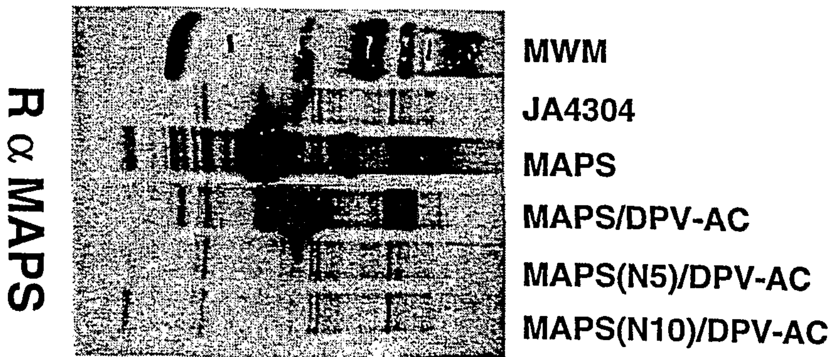


Fig. 3A SEQ ID NO:66 - TSA (i.e., MAPS) nucleic acid sequence

```
   1 ctgtacttta ttgccaccag ccagccatgt cctgcggtaa cgccaagatc aactctcccg
  61 cgccgtcctt cgaggaggtg gcgctcatgc ccaacggcag cttcaagaag atcagcctct
 121 cctcctacaa gggcaagtgg gtcgtgctct tcttctaccc gctcgacttt agcttcgtgt
 181 gcccgacaga ggtcatcgcg ttctccgaca gcgtgagtcg cttcaacgag ctcaactgcg
 241 aggtcctcgc gtgctcgata gacagcgagt acgcgcacct gcagtggacg ctgcaggacc
 301 gcaagaaggg cggcctcggg accatggcga tcccaatgct agccgacaag accaagagca
 361 tcgctcgttc ctacggcgtg ctggaggaga gccagggcgt ggcctaccgc ggtctcttca
 421 tcatcgaccc ccatggcatg ctgcgtcaga tcaccgtcaa tgacatgccg gtgggccgca
 481 gcgtggagga ggttctacgc ctgctggagg cttttcagtt cgtggagaag cacggcgagg
 541 tgtgccccgc gaactggaag aagggcgccc ccacgatgaa gccggaaccg aatgcgtctg
 601 tcgaaggata cttcagcaag cagtaaacct gtgagcgtcg caggagtcag tgtgacctca
 661 cccgcctctg ccagtgggtg cgagagggcg tgagggattg tgggaaggct gttggatatg
 721 atgcagacag cgatgaatgc aactcccaca cactggccct cctcagccct ctccacacag
 781 acacacgcac gcatgtgcgg tgcttctgct ccttcatttc ctgatgcggt ttctttacta
 841 tttatgtttt cccctgtttt ggtttcgcgg aatatggacg cacgaacgca gagagcagag
 901 gcgcgacaca cacacacggg gcgcacacgt gtaagcgacg gagcgcgaca cagaggggag
 961 aagccggaaa ggatatatga aatgaagatg gtgtcccgca cacacaggca cgcacacggg
1021 gcgaacgcgt ggcgcgtcct ttctatggtt tcgctgctgc tgttcgtgtt ctcgcttcgc
1081 tggttgcagt tccggctcgt gctgccctgc ctgccactct tggtccgtgt ctgtccgtgt
1141 ccgtgtccgt gtgggggggg gagagttttt cactcccccg gtcacctcta ctgtttatta
1201 tttatatgtt tatttatttc catattgact caattcattc cgtgcgaaag ccggcttgtt
1261 ccggcgtctt ttgacgaaca actgccctat cagctggtga tggccgtgta gtggactgcc
1321 atggcgttga cgggagcggg ggattagggt tcgattccgg agagggagcc tgagaaatag
1381 ctaccacttc tacggagggc agcaggcgcg caaattgccc aatgtcaaaa caaaacgatg
1441 aggcagcgaa aagaaataga gttgtcagtc catttggatt gtcatttcaa tgggggatat
1501 ttaaacccat ccaatatcga gtaacaattg gaggacaagt ctggtgccag cacccgcggt
1561 aattccagct ccaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
1621 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
1681 aaaaaaaaaa a
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Fig. 3B SEQ ID NO:67 - TSA (i.e., MAPS) amino acid sequence

```
                  MSCGNAKINSPAPSFEEVALMPNGSFKKISLSSYKGKWVVLFFY
PLDFSFVCPTEVIAFSDSVSRFNELNCEVLACSIDSEYAHLQWTLQDRKKGGLGTMAI
PMLADKTKSIARSYGVLEESQGVAYRGLFIIDPHGMLRQITVNDMPVGRSVEEVLRLL
EAFQFVEKHGEVCPANWKKGAPTMKPEPNASVEGYFSKQ
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Fig. 4 SEQ ID NOS:68 and 69
LeIF (i.e., LbeIF4A) nucleic acid and amino acid sequences

```
CCACTCTCTC GGTCGTCTGT CTCCCACGCG CGCACGCAGT TGATTTCCGC CTTCTTAAAC    60

GCTCTCTTTT TTTTTATTTT TCACCTGACC AACCGCACCA CGTCGGCCTC CATC ATG     117
                                                            Met
                                                              1

TCG CAG CAA GAC CGA GTT GCC CCA CAG GAC CAG GAC TCG TTC CTC GAC    165
Ser Gln Gln Asp Arg Val Ala Pro Gln Asp Gln Asp Ser Phe Leu Asp
            5                   10                  15

GAC CAG CCC GGC GTC CGC CCG ATC CCG TCC TTC GAT GAC ATG CCG TTG    213
Asp Gln Pro Gly Val Arg Pro Ile Pro Ser Phe Asp Asp Met Pro Leu
        20                  25                  30

CAC CAG AAC CTT CTG CGC GGC ATC TAC TCG TAC GGC TTC GAG AAA CCG    261
His Gln Asn Leu Leu Arg Gly Ile Tyr Ser Tyr Gly Phe Glu Lys Pro
    35                  40                  45

TCC AGC ATC CAG CAG CGC GCC ATC GCC CCC TTC ACG CGC GGC GGC GAC    309
Ser Ser Ile Gln Gln Arg Ala Ile Ala Pro Phe Thr Arg Gly Gly Asp
50                  55                  60                  65

ATC ATC GCG CAG GCG CAG TCC GGT ACC GGC AAG ACG GGC GCC TTC TCC    357
Ile Ile Ala Gln Ala Gln Ser Gly Thr Gly Lys Thr Gly Ala Phe Ser
                70                  75                  80

ATC GGC CTG CTG CAG CGC CTG GAC TTC CGC CAC AAC CTG ATC CAG GGC    405
Ile Gly Leu Leu Gln Arg Leu Asp Phe Arg His Asn Leu Ile Gln Gly
            85                  90                  95

CTC GTG CTC TCC CCG ACC CGC GAG CTG GCC CTG CAG ACG GCG GAG GTG    453
Leu Val Leu Ser Pro Thr Arg Glu Leu Ala Leu Gln Thr Ala Glu Val
        100                 105                 110

ATC AGC CGC ATC GGC GAG TTC CTG TCG AAC AGC GCG AAG TTC TGT GAG    501
Ile Ser Arg Ile Gly Glu Phe Leu Ser Asn Ser Ala Lys Phe Cys Glu
    115                 120                 125

ACC TTT GTG GGT GGC ACG CGC GTG CAG GAT GAC CTG CGC AAG CTG CAG    549
Thr Phe Val Gly Gly Thr Arg Val Gln Asp Asp Leu Arg Lys Leu Gln
130                 135                 140                 145

GCT GGC GTC GTC GTC GCC GTG GGG ACG CCG GGC CGC GTG TCC GAC GTG    597
Ala Gly Val Val Val Ala Val Gly Thr Pro Gly Arg Val Ser Asp Val
                150                 155                 160

ATC AAG CGC GGC GCG CTG CGC ACC GAG TCC CTG CGC GTG CTG GTG CTC    645
Ile Lys Arg Gly Ala Leu Arg Thr Glu Ser Leu Arg Val Leu Val Leu
            165                 170                 175

GAC GAG GCT GAT GAG ATG CTG TCT CAG GGC TTC GCG GAT CAG ATT TAC    693
Asp Glu Ala Asp Glu Met Leu Ser Gln Gly Phe Ala Asp Gln Ile Tyr
        180                 185                 190

GAG ATC TTC CGC TTC CTG CCG AAG GAC ATC CAG GTC GCG CTC TTC TCC    741
Glu Ile Phe Arg Phe Leu Pro Lys Asp Ile Gln Val Ala Leu Phe Ser
    195                 200                 205

GCC ACG ATG CCG GAG GAG GTG CTG GAG CTG ACA AAG AAG TTC ATG CGC    789
Ala Thr Met Pro Glu Glu Val Leu Glu Leu Thr Lys Lys Phe Met Arg
210                 215                 220                 225

GAC CCC GTA CGC ATT CTC GTG AAG CGC GAG AGC CTG ACG CTG GAG GGC    837
Asp Pro Val Arg Ile Leu Val Lys Arg Glu Ser Leu Thr Leu Glu Gly
                230                 235                 240

ATC AAG CAG TTC TTC ATC GCC GTC GAG GAG GAG CAC AAG CTG GAC ACG    885
Ile Lys Gln Phe Phe Ile Ala Val Glu Glu Glu His Lys Leu Asp Thr
            245                 250                 255

CTG ATG GAC CTG TAC GAG ACC GTG TCC ATC GCG CAG TCC GTC ATC TTC    933
Leu Met Asp Leu Tyr Glu Thr Val Ser Ile Ala Gln Ser Val Ile Phe
        260                 265                 270

GCC AAC ACC CGC CGC AAG GTG GAC TGG ATC GCC GAG AAG CTG AAT CAG    981
Ala Asn Thr Arg Arg Lys Val Asp Trp Ile Ala Glu Lys Leu Asn Gln
    275                 280                 285

AGC AAC CAC ACC GTC AGC AGC ATG CAC GCC GAG ATG CCC AAG AGC GAC   1029
Ser Asn His Thr Val Ser Ser Met His Ala Glu Met Pro Lys Ser Asp
```

Fig. 4 (Cont'd)

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290                 295                 300                 305

CGC GAG CGC GTC ATG AAC ACC TTC CGC AGC GGC AGC TCC CGC GTG CTC     1077
Arg Glu Arg Val Met Asn Thr Phe Arg Ser Gly Ser Ser Arg Val Leu
                310                 315                 320

GTA ACG ACC GAC CTC GTG GCC CGC GGC ATC GAC GTG CAC CAC GTG AAC     1125
Val Thr Thr Asp Leu Val Ala Arg Gly Ile Asp Val His His Val Asn
            325                 330                 335

ATC GTC ATC AAC TTC GAC CTG CCG ACG AAC AAG GAG AAC TAC CTG CAC     1173
Ile Val Ile Asn Phe Asp Leu Pro Thr Asn Lys Glu Asn Tyr Leu His
        340                 345                 350

CGC ATT GGC CGC GGC GGC CGC TAC GGC GTA AAG GGT GTT GCC ATC AAC     1221
Arg Ile Gly Arg Gly Gly Arg Tyr Gly Val Lys Gly Val Ala Ile Asn
    355                 360                 365

TTC GTG ACG GAG AAA GAC GTG GAG CTG CTG CAC GAG ATC GAG GGG CAC     1269
Phe Val Thr Glu Lys Asp Val Glu Leu Leu His Glu Ile Glu Gly His
370                 375                 380                 385

TAC CAC ACG CAG ATC GAT GAG CTC CCG GTG GAC TTT GCC GCC TAC CTC     1317
Tyr His Thr Gln Ile Asp Glu Leu Pro Val Asp Phe Ala Ala Tyr Leu
                390                 395                 400

GGC GAG TGA GCGGGCCCCT GCCCCCCTTC CCTGCCCCCC TCTCGCGACG             1366
Gly Glu

AGAGAACGCA CATCGTAACA CAGCCACGCG AACGATAGTA AGGGCGTGCG GCGGCGTTCC   1426

CCTCCTCCTG CCAGCGGCCC CCCTCCGCAG CGCTTCTCTT TTGAGAGGGG GGCAGGGGGA   1486

GGCGCTGCGC CTGGCTGGAT GTGTGCTTGA GCTTGCATTC CGTCAAGCAA GTGCTTTGTT   1546

TTAATTATGC GCGCCGTTTT GTTGCTCGTC CCTTTCGTTG GTGTTTTTTC GGCCGAAACG   1606

GCGTTTAAAG CA                                                       1618
```

Fig. 5 SEQ ID NOS:70 and 71
M15 (i.e., LmSTI1) nucleic acid and amino acid sequences

CAAGTGTCGA AGGACAGTGT TCNCCGTGTG AGATCGCCGG CTGTGCGTGT GAAGGCGGTG 6

CCATCGGANA AACAACACCG GTGGANCCGC AGGAAACCAT CTTTCTCCGC AGGTCTCTTT 12

TTGTTGTCGA TTGAGAGTGC NCCAAACCCT GCTGGTGCCC TTCTCACATA TCATGTTTTT 18

CGTTGTGCGC TCGCTTTGCC TTTCCTCTCC TTTCCCTCTC TTCCGTGGTG CCGTGTATAC 24

TTCTGGCACC CGCTACGTCA CTTCGCTGGT TTGAACAGAA CCACTGTGAA CACCCACGGG 30

CGATCGCACA CATACACATC CCTCACTCAC ACACACAGCT ACATCTATCC TACATAAAGC 36

TGAAAAAAAA GTCTACGAAC AATTTTGTTT TTACAGTGCG TTGCCGCACA TTTCTCCGTA 42

ATG GAC GCA ACT GAG CTG AAG AAC AAG GGG AAC GAA GAG TTC TCC GCC 46
Met Asp Ala Thr Glu Leu Lys Asn Lys Gly Asn Glu Gln Phe Ser Ala
1 5 10 15

GGC CGC TAT GTG GAG GCG GTG AAC TAC TTC TCA AAG GCG ATC CAG TTG 51
Gly Arg Tyr Val Glu Ala Val Asn Tyr Phe Ser Lys Ala Ile Gln Leu
20 25 30

GAT GAG CAG AAC AGT GTC CTC TAC AGC AAC CGC TCC GCC TGT TTT GCA 56
Asp Glu Gln Asn Ser Val Leu Tyr Ser Asn Arg Ser Ala Cys Phe Ala
35 40 45

GCC ATG CAG AAA TAC AAG GAC GCG CTG GAC GAC GCC GAC AAG TGC ATC 61
Ala Met Gln Lys Tyr Lys Asp Ala Leu Asp Asp Ala Asp Lys Cys Ile
50 55 60

TCG ATC AAG CCG AAT TGG GCC AAG GGC TAC GTG CGC CGA GGA GCA GCT 66
Ser Ile Lys Pro Asn Trp Ala Lys Gly Tyr Val Arg Arg Gly Ala Ala
65 70 75 80

CTC CAT GGC ATG CGC CGC TAC GAC GAT GCC ATT GCC GCG TAT GAA AAG 70
Leu His Gly Met Arg Arg Tyr Asp Asp Ala Ile Ala Ala Tyr Glu Lys
85 90 95

GGG CTC AAG GTG GAC CCT TCC AAC AGC GGC TGC GCG CAG GGC GTG AAG 75
Gly Leu Lys Val Asp Pro Ser Asn Ser Gly Cys Ala Gln Gly Val Lys
100 105 110

GAC GTG CAG GTA GCC AAG GCC CGC GAA GCA CGT GAC CCC ATC GCT CGC 80
Asp Val Gln Val Ala Lys Ala Arg Glu Ala Arg Asp Pro Ile Ala Arg
115 120 125

GTC TTC ACC CCG GAG GCG TTC CGC AAG ATC CAA GAG AAT CCC AAG CTG 85
Val Phe Thr Pro Glu Ala Phe Arg Lys Ile Gln Glu Asn Pro Lys Leu
130 135 140

TCT CTA CTT ATG CTG CAG CCG GAC TAC GTG AAG ATG GTA GAC ACC GTC 90
Ser Leu Leu Met Leu Gln Pro Asp Tyr Val Lys Met Val Asp Thr Val
145 150 155 160

ATC CGC GAC CCT TCG CAG GGC CGG CTG TAC ATG GAA GAC CAG CGC TTT 94
Ile Arg Asp Pro Ser Gln Gly Arg Leu Tyr Met Glu Asp Gln Arg Phe
165 170 175

GCC CTG ACG CTC ATG TAC CTG AGC GGA ATG AAG ATT CCC AAC GAT GGT 99
Ala Leu Thr Leu Met Tyr Leu Ser Gly Met Lys Ile Pro Asn Asp Gly
180 185 190

GAT GGC GAG GAG GAG GAA CGT CCG TCT GCG AAG GCG GCA GAG ACA GCG 104
Asp Gly Glu Glu Glu Glu Arg Pro Ser Ala Lys Ala Ala Glu Thr Ala
195 200 205

AAG CCA AAA GAG GAG AAG CCT CTC ACC GAC AAC GAG AAG GAG GCC CTG 109
Lys Pro Lys Glu Glu Lys Pro Leu Thr Asp Asn Glu Lys Glu Ala Leu
210 215 220

GCG CTC AAG GAG GAG GGC AAC AAG CTG TAC CTC TCG AAG AAG TTT GAG 114
Ala Leu Lys Glu Glu Gly Asn Lys Leu Tyr Leu Ser Lys Lys Phe Glu
225 230 235 240

GAG GCG CTG ACC AAG TAC CAA GAG GCG CAG GTG AAA GAC CCC AAC AAC 118
Glu Ala Leu Thr Lys Tyr Gln Glu Ala Gln Val Lys Asp Pro Asn Asn

Fig. 5 (Cont'd)

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ACT TTA TAC ATT CTG AAC GTG TCG GCC GTG TAC TTC GAG CAG GGT GAC     123
Thr Leu Tyr Ile Leu Asn Val Ser Ala Val Tyr Phe Glu Gln Gly Asp
            260                 265                 270

TAC GAC AAG TGC ATC GCC GAG TGC GAG CAC GGT ATC GAG CAC GGT CGC     128
Tyr Asp Lys Cys Ile Ala Glu Cys Glu His Gly Ile Glu His Gly Arg
        275                 280                 285

GAG AAC CAC TGC GAC TAC ACA ATC ATT GCG AAG CTC ATG ACC CGG AAC     133
Glu Asn His Cys Asp Tyr Thr Ile Ile Ala Lys Leu Met Thr Arg Asn
    290                 295                 300

GCC TTG TGC CTC CAG AGG CAG AGG AAG TAC GAG GCT GCT ATC GAC CTT     138
Ala Leu Cys Leu Gln Arg Gln Arg Lys Tyr Glu Ala Ala Ile Asp Leu
305                 310                 315                 320

TAC AAG CGC GCC CTT GTC GAG TGG CGT AAC CCT GAC ACC CTC AAG AAG     142
Tyr Lys Arg Ala Leu Val Glu Trp Arg Asn Pro Asp Thr Leu Lys Lys
                325                 330                 335

CTG ACG GAG TGC GAG AAG GAG CAC CAA AAG GCG GTG GAG GAA GCC TAC     147
Leu Thr Glu Cys Glu Lys Glu His Gln Lys Ala Val Glu Glu Ala Tyr
            340                 345                 350

ATC GAT CCT GAG ATC GCG AAG CAG AAG AAA GAC GAA GGT AAC CAG TAC     152
Ile Asp Pro Glu Ile Ala Lys Gln Lys Lys Asp Glu Gly Asn Gln Tyr
        355                 360                 365

TTC AAG GAG GAT AAG TTC CCC GAG GCC GTG GCA GCG TAC ACG GAG GCC     157
Phe Lys Glu Asp Lys Phe Pro Glu Ala Val Ala Ala Tyr Thr Glu Ala
    370                 375                 380

ATC AAG CGC AAC CCT GCC GAG CAC ACC TCC TAC AGC AAT CGC GCG GCC     162
Ile Lys Arg Asn Pro Ala Glu His Thr Ser Tyr Ser Asn Arg Ala Ala
385                 390                 395                 400

GCG TAC ATC AAG CTT GGA GCC TTC AAC GAC GCC CTC AAG GAC GCG GAG     166
Ala Tyr Ile Lys Leu Gly Ala Phe Asn Asp Ala Leu Lys Asp Ala Glu
                405                 410                 415

AAG TGC ATT GAG CTG AAG CCC GAC TTT GTT AAG GGC TAC GCG CGC AAG     171
Lys Cys Ile Glu Leu Lys Pro Asp Phe Val Lys Gly Tyr Ala Arg Lys
            420                 425                 430

GGT CAT GCT TAC TTT TGG ACC AAG CAG TAC AAC CGC GCG CTG CAG GCG     176
Gly His Ala Tyr Phe Trp Thr Lys Gln Tyr Asn Arg Ala Leu Gln Ala
        435                 440                 445

TAC GAT GAG GGC CTC AAG GTG GAC CCG AGC AAT GCG GAC TGC AAG GAT     181
Tyr Asp Glu Gly Leu Lys Val Asp Pro Ser Asn Ala Asp Cys Lys Asp
    450                 455                 460

GGG CGG TAT CGC ACA ATC ATG AAG ATT CAG GAG ATG GCA TCT GGC CAA     186
Gly Arg Tyr Arg Thr Ile Met Lys Ile Gln Glu Met Ala Ser Gly Gln
465                 470                 475                 480

TCC GCG GAT GGC GAC GAG GCG GCG CGC CGG GCC ATG GAC GAT CCT GAA     190
Ser Ala Asp Gly Asp Glu Ala Ala Arg Arg Ala Met Asp Asp Pro Glu
                485                 490                 495

ATC GCG GCA ATC ATG CAA GAT AGC TAC ATG CAA CTA GTG TTG AAG GAG     195
Ile Ala Ala Ile Met Gln Asp Ser Tyr Met Gln Leu Val Leu Lys Glu
            500                 505                 510

ATG CAG AAC GAT CCC ACG CGC ATT CAG GAG TAC ATG AAG GAC TCC GGG     200
Met Gln Asn Asp Pro Thr Arg Ile Gln Glu Tyr Met Lys Asp Ser Gly
        515                 520                 525

ATC TCA TCG AAG ATC AAC AAG CTG ATT TCA GCT GGC ATC ATT CGT TTT     205
Ile Ser Ser Lys Ile Asn Lys Leu Ile Ser Ala Gly Ile Ile Arg Phe
    530                 535                 540

GGT CAG TAGACTTCTA CGCTGCCTCA TCTTTTCCGT GTCTTTGCGT CGGCGGGTAT      210
Gly Gln
545

CGTAAAGCAC AATAAAGCAG CGATTCACAT GCACGAGTAA AGTGCTGCGC CTCTCAAACA   216
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Fig. 5 (Cont'd)

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CGACGTCGAG GCTGTGGTGC AGATGCGCGT CCTGCATGAA GGTAGTGAAG AGGAAAGTAA   222
GGGATGTTGT TTGTGGGCCT TCGTGGCTGC GCACACACCT CTTATCTCCT TCGCTTGGTA   228
CCTTCTCCCT TTTTCGTCTT CACCCCCCTT TCTCTTCTCA CGCTCTCCCT GGCGCGGTGG   234
TGCAACGATT TCGTTTTATT TACGTCTGTG TAGCTCCTCT ATTCAACGGT GCGATGACGC   240
TAACGAAGCT GGCCTGTATT CGGCTAAGGC GAAGGCAAAA GACTAGGAGG GGGGGGGGAA   246
GGAGACGGCG TGACCATCAC TGCGAAGAAA CAAGCCGAAG AAAAGGCCCC GAACGCCTGC   252
ATTTCCGCGC GCCCTCGCCC GCCTTCCTTC CTTCCTTCGC TCTCTCTCTC TCTCTCTCTC   258
GCTATCTTCT CAACGGAGAC ATGAAAGGCG TTTGTTAGGA AAAGAGGGGG GGGGGAAGAG   264
TGGGACGACG CGCTGCGTCT TTTGGGCACT GGTCACGTGC GTCACCCTCT TTTTTTATCT   270
CTATTGGCAC TGTCTTGTTT CTTTTCCCTT TCCTATCATA CGCGTCTCGC AAACGACTCC   276
GCGCTGAGCA GCCATGTGCT GCGGCGTGGA GGAAGTACAC AGACATCACG GATGCATATG   282
TGCGCGTCCG TGTACGCGCT TGTATGGGGC TTCTAACAGC GCCTGTGTGT GTTTGTGTGT   288
GTGTGTGTGT GTGTGTCTGT GTATTTCGAG CGTCTGTATG CTATTCTATT AAGCACCGAA   294
GAAGAGACAC ACACGACAGC GAAGGAGATG GTGTCGGCTT TTCGGCTAAT CACTCCCTTC   300
CATAGCTTCT CTGAAGGAGG CTCTCTTCCA GAGGAATAGA CTGCAGATGG GGTCCACGTT   306
TATCTGAGGA GTCAACGGAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA   312
CTCGAG                                                              313
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Fig. 6 SEQ ID NOS:72 and 73
6H (i.e., Lbhsp83) nucleic acid sequence

CGCGGTGGCG GCCGCTCTAG AACTAGTGGA TCCCCCGGGC TGCAGGAATT CGGCACGAGA 60

G AGC CTG ACG GAC CCG GCG GTG CTG GGC GAG GAG ACT CAC CTG CGC 106
Ser Leu Thr Asp Pro Ala Val Leu Gly Glu Glu Thr His Leu Arg
180 185 190

GTC CGC GTG GTG CCG GAC AAG GCG AAC AAG ACG CTG ACG GTG GAG GAT 154
Val Arg Val Val Pro Asp Lys Ala Asn Lys Thr Leu Thr Val Glu Asp
195 200 205

AAC GGC ATC GGC ATG ACC AAG GCG GAC CTC GTG AAC AAT CTG GGC ACG 202
Asn Gly Ile Gly Met Thr Lys Ala Asp Leu Val Asn Asn Leu Gly Thr
210 215 220

ATC GCG CGC TCC GGC ACG AAG GCT TTC ATG GAG GCA CTG GAG GCC GGC 250
Ile Ala Arg Ser Gly Thr Lys Ala Phe Met Glu Ala Leu Glu Ala Gly
225 230 235

GGC GAC ATG AGC ATG ATC GGC CAG TTC GGT GTC GGC TTC TAC TCC GCG 298
Gly Asp Met Ser Met Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala
240 245 250

TAC CTT GTG GCG GAC CGC GTG ACG GTG GTG TCG AAG AAC AAC TCG GAC 346
Tyr Leu Val Ala Asp Arg Val Thr Val Val Ser Lys Asn Asn Ser Asp
255 260 265 270

GAG GCG TAC TGG GAA TCG TCT GCG GGG GGC ACG TTC ACC ATC ACG AGC 394
Glu Ala Tyr Trp Glu Ser Ser Ala Gly Gly Thr Phe Thr Ile Thr Ser
275 280 285

GTG CAG GAG TCG GAC ATG AAG CGC GGC ACG AGT ACA ACG CTG CAC CTA 442
Val Gln Glu Ser Asp Met Lys Arg Gly Thr Ser Thr Thr Leu His Leu
290 295 300

AAG GAG GAC CAG CAG GAG TAC CTG GAG GAG CGC CGG GTG AAG GAG CTG 490
Lys Glu Asp Gln Gln Glu Tyr Leu Glu Glu Arg Arg Val Lys Glu Leu
305 310 315

ATC AAG AAG CAC TCC GAG TTC ATC GGC TAC GAC ATC GAG CTG ATG GTG 538
Ile Lys Lys His Ser Glu Phe Ile Gly Tyr Asp Ile Glu Leu Met Val
320 325 330

GAG AAG ACG GCG GAG AAG GAG GTG ACG GAC GAG GAC GAG GAG GAG GAC 586
Glu Lys Thr Ala Glu Lys Glu Val Thr Asp Glu Asp Glu Glu Glu Asp
335 340 345 350

GAG TCG AAG AAG AAG TCC TGC GGG GAC GAG GGC GAG CCG AAG GTG GAG 634
Glu Ser Lys Lys Lys Ser Cys Gly Asp Glu Gly Glu Pro Lys Val Glu
355 360 365

GAG GTG ACG GAG GGC GGC GAG GAC AAG AAG AAG AAG ACG AAG AAG GTG 682
Glu Val Thr Glu Gly Gly Glu Asp Lys Lys Lys Lys Thr Lys Lys Val
370 375 380

AAG GAG GTG AAG AAG ACG TAC GAG GTC AAG AAC AAG CAC AAG CCG CTC 730
Lys Glu Val Lys Lys Thr Tyr Glu Val Lys Asn Lys His Lys Pro Leu
385 390 395

TGG ACG CGC GAC ACG AAG GAC GTG ACG AAG GAG GAG TAC GCG GCC TTC 778
Trp Thr Arg Asp Thr Lys Asp Val Thr Lys Glu Glu Tyr Ala Ala Phe
400 405 410

TAC AAG GCC ATC TCC AAC GAC TGG GAG GAC ACG GCG GCG ACG AAG CAC 826
Tyr Lys Ala Ile Ser Asn Asp Trp Glu Asp Thr Ala Ala Thr Lys His
415 420 425 430

TTC TCG GTG GAG GGC CAG CTG GAG TTC CGC GCG ATC GCG TTC GTG CCG 874
Phe Ser Val Glu Gly Gln Leu Glu Phe Arg Ala Ile Ala Phe Val Pro
435 440 445

AAG CGC GCG CCG TTC GAC ATG TTC GAG CCG AAC AAG AAG CGC AAC AAC 922
Lys Arg Ala Pro Phe Asp Met Phe Glu Pro Asn Lys Lys Arg Asn Asn
450 455 460

ATC AAG CTG TAC GTG CGC CGC GTG TTC ATC ATG GAC AAC TGC GAG GAC 970
Ile Lys Leu Tyr Val Arg Arg Val Phe Ile Met Asp Asn Cys Glu Asp
465 470 475

CTG TGC CCG GAC TGG CTC GGC TTC GTG AAG GGC GTC GTG GAC AGC GAG 1018
Leu Cys Pro Asp Trp Leu Gly Phe Val Lys Gly Val Val Asp Ser Glu
480 485 490

GAC CTG CCG CTG AAC ATC TCG CGC GAG AAC CTG CAG CAG AAC AAG ATC 1066
Asp Leu Pro Leu Asn Ile Ser Arg Glu Asn Leu Gln Gln Asn Lys Ile
495 500 505 510

CTG AAG GTG ATC CGC AAG AAC ATC GTG AAG AAG TGC CTG GAG CTG TTC 1114
Leu Lys Val Ile Arg Lys Asn Ile Val Lys Lys Cys Leu Glu Leu Phe
515 520 525

Fig. 6 (Cont'd)

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GAA GAG ATA GCG GAG AAC AAG GAG GAC TAC AAG CAG TTC TAC GAG CAG      1162
Glu Glu Ile Ala Glu Asn Lys Glu Asp Tyr Lys Gln Phe Tyr Glu Gln
            530                 535                 540

TTC GGC AAG AAC ATC AAG CTG GGC ATC CAC GAG GAC ACG GCG AAC CGC      1210
Phe Gly Lys Asn Ile Lys Leu Gly Ile His Glu Asp Thr Ala Asn Arg
        545                 550                 555

AAG AAG CTG ATG GAG TTG CTG CGC TTC TAC AGC ACC GAG TCG GGG GAG      1258
Lys Lys Leu Met Glu Leu Leu Arg Phe Tyr Ser Thr Glu Ser Gly Glu
    560                 565                 570

GAG ATG ACG ACA CTG AAG GAC TAC GTG ACG CGC ATG AAG CCG GAG CAG      1306
Glu Met Thr Thr Leu Lys Asp Tyr Val Thr Arg Met Lys Pro Glu Gln
575                 580                 585                 590

AAG TCG ATC TAC TAC ATC ACT GGC GAC AGC AAG AAG AAG CTG GAG TCG      1354
Lys Ser Ile Tyr Tyr Ile Thr Gly Asp Ser Lys Lys Lys Leu Glu Ser
                595                 600                 605

TCG CCG TTC ATC GAG AAG GCG AGA CGC TGC GGG CTC GAG GTG CTG TTC      1402
Ser Pro Phe Ile Glu Lys Ala Arg Arg Cys Gly Leu Glu Val Leu Phe
            610                 615                 620

ATG ACG GAG CCG ATC GAC GAG TAC GTG ATG CAG CAG GTG AAG GAC TTC      1450
Met Thr Glu Pro Ile Asp Glu Tyr Val Met Gln Gln Val Lys Asp Phe
        625                 630                 635

GAG GAC AAG AAG TTC GCG TGC CTG ACG AAG GAA GGC GTG CAC TTC GAG      1498
Glu Asp Lys Lys Phe Ala Cys Leu Thr Lys Glu Gly Val His Phe Glu
    640                 645                 650

GAG TCC GAG GAG GAG AAG AAG CAG CGC GAG GAG AAG AAG GCG GCG TGC      1546
Glu Ser Glu Glu Glu Lys Lys Gln Arg Glu Glu Lys Lys Ala Ala Cys
655                 660                 665                 670

GAG AAG CTG TGC AAG ACG ATG AAG GAG GTG CTG GGC GAC AAG GTG GAG      1594
Glu Lys Leu Cys Lys Thr Met Lys Glu Val Leu Gly Asp Lys Val Glu
                675                 680                 685

AAG GTG ACC GTG TCG GAG CGC CTG TTG ACG TCG CCG TGC ATC CTG GTG      1642
Lys Val Thr Val Ser Glu Arg Leu Leu Thr Ser Pro Cys Ile Leu Val
            690                 695                 700

ACG TCG GAG TTT GGG TGG TCG GCG CAC ATG GAA CAG ATC ATG CGC AAC      1690
Thr Ser Glu Phe Gly Trp Ser Ala His Met Glu Gln Ile Met Arg Asn
        705                 710                 715

CAG GCG CTG CGC GAC TCC AGC ATG GCG CAG TAC ATG GTG TCC AAG AAG      1738
Gln Ala Leu Arg Asp Ser Ser Met Ala Gln Tyr Met Val Ser Lys Lys
    720                 725                 730

ACG ATG GAG GTG AAC CCC GAC CAC CCC ATC ATC AAG GAG CTG CGC CGC      1786
Thr Met Glu Val Asn Pro Asp His Pro Ile Ile Lys Glu Leu Arg Arg
735                 740                 745                 750

CGC GTG GAG GCG GAC GAG AAC GAC AAG GCC GTG AAG GAC CTC GTC TTC      1834
Arg Val Glu Ala Asp Glu Asn Asp Lys Ala Val Lys Asp Leu Val Phe
                755                 760                 765

CTG CTC TTC GAC ACG TCG CTG CTC ACG TCC GGC TTC CAG CTG GAT GAC      1882
Leu Leu Phe Asp Thr Ser Leu Leu Thr Ser Gly Phe Gln Leu Asp Asp
            770                 775                 780

CCC ACC GGC TAC GCC GAG CGC ATC AAC CGC ATG ATC AAG CTC GGC CTG      1930
Pro Thr Gly Tyr Ala Glu Arg Ile Asn Arg Met Ile Lys Leu Gly Leu
        785                 790                 795

TCG CTC GAC GAG GAG GAG GAG GAG GTC GCC GAG GCG CCG CCG GCC GAG      1978
Ser Leu Asp Glu Glu Glu Glu Glu Val Ala Glu Ala Pro Pro Ala Glu
    800                 805                 810

GCA GCC CCC GCG GAG GTC ACC GCC GGC ACC TCC AGC ATG GAG CAG GTG      2026
Ala Ala Pro Ala Glu Val Thr Ala Gly Thr Ser Ser Met Glu Gln Val
815                 820                 825                 830

GAC TGAGCCGGTA A                                                     2040
Asp
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