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ABSTRACT

The present invention relates to nucleic acid vaccines encoding multiple CTL and HTL epitopes and MHC targeting sequences.

EXPRESSION VECTORS FOR STIMULATING AN IMMUNE RESPONSE AND METHODS OF USING THE SAME

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

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FIELD OF THE INVENTION

15 The present invention relates to nucleic acid vaccines encoding multiple CTL and HTL epitopes and MHC targeting sequences.

BACKGROUND OF THE INVENTION

20 Vaccines are of fundamental importance in modern medicine and have been highly effective in combating certain human diseases. However, despite the successful implementation of vaccination programs that have greatly limited or virtually eliminated several debilitating human diseases, there are a number of diseases that affect millions worldwide for which effective vaccines have not been developed.

25 Major advances in the field of immunology have led to a greater understanding of the mechanisms involved in the immune response and have provided insights into developing new vaccine strategies (Kuby, *Immunology*, 443-457 (3rd ed., 1997)). These new vaccine strategies have taken advantage of knowledge gained regarding the mechanisms by which foreign material, termed antigen, is recognized by the immune system and eliminated from the
30 organism. An effective vaccine is one that elicits an immune response to an antigen of interest.

 Specialized cells of the immune system are responsible for the protective activity required to combat diseases. An immune response involves two major groups of cells, lymphocytes, or white blood cells, and antigen-presenting cells. The purpose of

these immune response cells is to recognize foreign material, such as an infectious organism or a cancer cell, and remove that foreign material from the organism.

Two major types of lymphocytes mediate different aspects of the immune response. B cells display on their cell surface specialized proteins, called antibodies, that bind specifically to foreign material, called antigens. Effector B cells produce soluble forms of the antibody, which circulate throughout the body and function to eliminate antigen from the organism. This branch of the immune system is known as the humoral branch. Memory B cells function to recognize the antigen in future encounters by continuing to express the membrane-bound form of the antibody.

A second major type of lymphocyte is the T cell. T cells also have on their cell surface specialized proteins that recognize antigen but, in contrast to B cells, require that the antigen be bound to a specialized membrane protein complex, the major histocompatibility complex (MHC), on the surface of an antigen-presenting cell. Two major classes of T cells, termed helper T lymphocytes ("HTL") and cytotoxic T lymphocytes ("CTL"), are often distinguished based on the presence of either CD4 or CD8 protein, respectively, on the cell surface. This branch of the immune system is known as the cell-mediated branch.

The second major class of immune response cells are cells that function in antigen presentation by processing antigen for binding to MHC molecules expressed in the antigen presenting cells. The processed antigen bound to MHC molecules is transferred to the surface of the cell, where the antigen-MHC complex is available to bind to T cells.

MHC molecules can be divided into MHC class I and class II molecules and are recognized by the two classes of T cells. Nearly all cells express MHC class I molecules, which function to present antigen to cytotoxic T lymphocytes. Cytotoxic T lymphocytes typically recognize antigen bound to MHC class I. A subset of cells called antigen-presenting cells express MHC class II molecules. Helper T lymphocytes typically recognize antigen bound to MHC class II molecules. Antigen-presenting cells include dendritic cells, macrophages, B cells, fibroblasts, glial cells, pancreatic beta cells, thymic epithelial cells, thyroid epithelial cells and vascular endothelial cells. These antigen-presenting cells generally express both MHC class I and class II molecules. Also, B cells function as both antibody-producing and antigen-presenting cells.

Once a helper T lymphocyte recognizes an antigen-MHC class II complex on the surface of an antigen-presenting cell, the helper T lymphocyte becomes activated

and produces growth factors that activate a variety of cells involved in the immune response, including B cells and cytotoxic T lymphocytes. For example, under the influence of growth factors expressed by activated helper T lymphocytes, a cytotoxic T lymphocyte that recognizes an antigen-MHC class I complex becomes activated. CTLs
 5 monitor and eliminate cells that display antigen specifically recognized by the CTL, such as infected cells or tumor cells. Thus, activation of helper T lymphocytes stimulates the activation of both the humoral and cell-mediated branches of the immune system.

An important aspect of the immune response, in particular as it relates to vaccine efficacy, is the manner in which antigen is processed so that it can be recognized
 10 by the specialized cells of the immune system. Distinct antigen processing and presentation pathways are utilized. The one is a cytosolic pathway, which results in the antigen being bound to MHC class I molecules. An alternative pathway is an endoplasmic reticulum pathway, which bypasses the cytosol. Another is an endocytic pathway, which results in the antigen being bound to MHC class II molecules. Thus, the
 15 cell surface presentation of a particular antigen by a MHC class II or class I molecule to a helper T lymphocyte or a cytotoxic T lymphocyte, respectively, is dependent on the processing pathway for that antigen.

The cytosolic pathway processes endogenous antigens that are expressed inside the cell. The antigen is degraded by a specialized protease complex in the cytosol
 20 of the cell, and the resulting antigen peptides are transported into the endoplasmic reticulum, an organelle that processes cell surface molecules. In the endoplasmic reticulum, the antigen peptides bind to MHC class I molecules, which are then transported to the cell surface for presentation to cytotoxic T lymphocytes of the immune system.

25 Antigens that exist outside the cell are processed by the endocytic pathway. Such antigens are taken into the cell by endocytosis, which brings the antigens into specialized vesicles called endosomes and subsequently to specialized vesicles called lysosomes, where the antigen is degraded by proteases into antigen peptides that bind to MHC class II molecules. The antigen peptide-MHC class II molecule complex is then
 30 transported to the cell surface for presentation to helper T lymphocytes of the immune system.

A variety of factors must be considered in the development of an effective vaccine. For example, the extent of activation of either the humoral or cell-mediated branch of the immune system can determine the effectiveness of a vaccine against a

particular disease. Furthermore, the development of immunologic memory by inducing memory-cell formation can be important for an effective vaccine against a particular disease (Kuby, *supra*). For example, protection from infectious diseases caused by pathogens with short incubation periods, such as influenza virus, requires high levels of neutralizing antibody generated by the humoral branch because disease symptoms are already underway before memory cells are activated. Alternatively, protection from infectious diseases caused by pathogens with long incubation periods, such as polio virus, does not require neutralizing antibodies at the time of infection but instead requires memory B cells that can generate neutralizing antibodies to combat the pathogen before it is able to infect target tissues. Therefore, the effectiveness of a vaccine at preventing or ameliorating the symptoms of a particular disease depends on the type of immune response generated by the vaccine.

Many traditional vaccines have relied on intact pathogens such as attenuated or inactivated viruses or bacteria to elicit an immune response. However, these traditional vaccines have advantages and disadvantages, including reversion of an attenuated pathogen to a virulent form. The problem of reversion of an attenuated vaccine has been addressed by the use of molecules of the pathogen rather than the whole pathogen. For example, immunization approaches have begun to incorporate recombinant vector vaccines and synthetic peptide vaccines (Kuby, *supra*). Recently, DNA vaccines have also been used (Donnelly *et al.*, *Annu. Rev. Immunol.* 15:617-648 (1997)). The use of molecules of a pathogen provides safe vaccines that circumvent the potential for reversion to a virulent form of the vaccine.

The targeting of antigens to MHC class II molecules to activate helper T lymphocytes has been described using lysosomal targeting sequences, which direct antigens to lysosomes, where the antigen is digested by lysosomal proteases into antigen peptides that bind to MHC class II molecules (U.S. Patent No. 5,633,234; Thomson *et al.*, *J. Virol.* 72:2246-2252 (1998)). It would be advantageous to develop vaccines that deliver multiple antigens while exploiting the safety provided by administering individual epitopes of a pathogen rather than a whole organism. In particular, it would be advantageous to develop vaccines that effectively target antigens to MHC class II molecules for activation of helper T lymphocytes.

Several studies also point to the crucial role of cytotoxic T cells in both production and eradication of infectious diseases and cancer by the immune system

(Byrne *et al.*, *J. Immunol.* 51:682 (1984); McMichael *et al.*, *N. Engl. J. Med.* 309:13 (1983)). Recombinant protein vaccines do not reliably induce CTL responses, and the use of otherwise immunogenic vaccines consisting of attenuated pathogens in humans is hampered, in the case of several important diseases, by overriding safety concerns. In the case of diseases such as HIV, HBV, HCV, and malaria, it appears desirable not only to induce a vigorous CTL response, but also to focus the response against highly conserved epitopes in order to prevent escape by mutation and overcome variable vaccine efficacy against different isolates of the target pathogen.

Induction of a broad response directed simultaneously against multiple epitopes also appears to be crucial for development of efficacious vaccines. HIV infection is perhaps the best example where an infected host may benefit from a multispecific response. Rapid progression of HIV infection has been reported in cases where a narrowly focused CTL response is induced whereas nonprogressors tend to show a broader specificity of CTLs (Goulder *et al.*, *Nat. Med.* 3:212 (1997); Borrow *et al.*, *Nat. Med.* 3:205 (1997)). The highly variable nature of HIV CTL epitopes resulting from a highly mutating genome and selection by CTL responses directed against only a single or few epitopes also supports the need for broad epitope CTL responses (McMichael *et al.*, *Annu. Rev. Immunol.* 15:271 (1997)).

One potential approach to induce multispecific responses against conserved epitopes is immunization with a minigene plasmid encoding the epitopes in a string-of-beads fashion. Induction of CTL, HTL, and B cell responses in mice by minigene plasmids have been described by several laboratories using constructs encoding as many as 11 epitopes (An *et al.*, *J. Virol.* 71:2292 (1997); Thomson *et al.*, *J. Immunol.* 157:822 (1996); Whitton *et al.*, *J. Virol.* 67:348 (1993); Hanke *et al.*, *Vaccine* 16:426 (1998); Vitiello *et al.*, *Eur. J. Immunol.* 27:671-678 (1997)). Minigenes have been delivered *in vivo* by infection with recombinant adenovirus or vaccinia, or by injection of purified DNA via the intramuscular or intradermal route (Thomson *et al.*, *J. Immunol.* 160:1717 (1998); Toes *et al.*, *Proc. Natl. Acad. Sci. USA* 94:14660 (1997)).

Successful development of minigene DNA vaccines for human use will require addressing certain fundamental questions dealing with epitope MHC affinity, optimization of constructs for maximum *in vivo* immunogenicity, and development of assays for testing *in vivo* potency of multi-epitope minigene constructs. Regarding MHC binding affinity of epitopes, it is not currently known whether both high and low affinity epitopes can be included within a single minigene construct, and what ranges of peptide

affinity are permissible for CTL induction *in vivo*. This is especially important because dominant epitopes can vary in their affinity and because it might be important to be able to deliver mixtures of dominant and subdominant epitopes that are characterized by high and low MHC binding affinities.

5 With respect to minigene construct optimization for maximum immunogenicity *in vivo*, conflicting data exists regarding whether the exact position of the epitopes in a given construct or the presence of flanking regions, helper T cell epitopes, and signal sequences might be crucial for CTL induction (Del Val *et al.*, *Cell* 66:1145 (1991); Bergmann *et al.*, *J. Virol.* 68:5306 (1994); Thomson *et al.*, *Proc. Natl.* 10 *Acad. Sci. USA* 92:5845 (1995); Shirai *et al.*, *J. Infect. Dis.* 173:24 (1996); Rahemtulla *et al.*, *Nature* 353:180 (1991); Jennings *et al.*, *Cell. Immunol.* 133:234 (1991); Anderson *et al.*, *J. Exp. Med.* 174:489 (1991); Uger *et al.*, *J. Immunol.* 158:685 (1997)). Finally, regarding development of assays that allow testing of human vaccine candidates, it should be noted that, to date, all *in vivo* immunogenicity data of multi-epitope minigene plasmids 15 have been performed with murine class I MHC-restricted epitopes. It would be advantageous to be able to test the *in vivo* immunogenicity of minigenes containing human CTL epitopes in a convenient animal model system.

 Thus, there exists a need to develop methods to effectively deliver a variety of HTL (helper T lymphocyte) and CTL (cytotoxic T lymphocyte) antigens to 20 stimulate an immune response. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

Various embodiments of this invention provide an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding two or more heterologous peptide epitopes, wherein the heterologous peptide epitopes comprise two class II restricted helper T lymphocyte (HTL) human immunodeficiency virus (HIV) peptide epitopes or a class I restricted cytotoxic T lymphocyte (CTL) HIV peptide epitope and a universal class II restricted (HTL) peptide epitope, wherein at least one of said class II restricted (HTL) HIV peptide epitopes is selected from the group consisting of: AETFYVDGAANRETK, EKVYLA WVP AHKGIG, EVNIVTDSQYALGII, FRKYTAFTIPSINNE, GAVVIQD NSDIKVVP, GEIYKRWIILGLNKI, HSNWRAMASDFNLPP, IKQFINMWQE V GKAMY, KRWIILGLNKIVRMY, KTAVQMAVFIHNFKR, KVYLA WVP AHKGIGG, QGQMVHQAISPRTL N, QHLLQLTVWGIKQLQ, QKQITKIQNFRVYYR, SPAIFQSSMTKILEP, WAGIKQEF GIPYNPQ, WEFVNTPLVLKLWYQ, and YRKILRQRKIDRLID; and further wherein said class I restricted (CTL) HIV peptide epitope is selected from the group consisting of: AIFQSSMTK, AVFIHNFKR, CPKVSFEPI, FPISPIETV, FPVRPQVPL, HPVHAGPIA, ILKEPVHGV, IPIHYCAPA, IPYNPQSQGVV, KIQNFRVYYR, KLTPLCVTL, KLVGKLNWA, KVYLA WVP AHK, LTFGWCFKL, MASDFNLPPV, MAVFIHNFK, MTNNPPIPV, QMAVFIHNFK, QVPLRPMTYK, RILQQLLFI, TTLFCASDAK, TVYYGVPVWK, VLAEAMSQV, VPLQLPPL, VTIKIGGQLK, VTVYYGVPVWK, and YPLASLRSLF. This invention also provides the use of such an expression vector for inducing an immune response *in vivo* in a mammalian subject as well as for preparation of a medicament for such use. Also provided are compositions comprising a pharmaceutically acceptable carrier and the aforementioned expression vector, for use in inducing an immune response *in vivo* in a mammalian subject.

Various embodiments of this invention provide a composition comprising a pharmaceutically acceptable carrier and an expression vector for use in inducing an immune response *in vivo* in a mammalian subject, wherein the expression vector comprises a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding a heterologous human class II restricted helper T lymphocyte (HTL) human

immunodeficiency virus (HIV) peptide epitope, wherein said HIV peptide class II restricted (HTL) HIV peptide epitope is selected from the group consisting of: AETFYVDGAANRETK, EKVYLAWVPAHKGIG, EVNIVTDSQYALGII, FRKYTAFTIPSINNE, GAVVIQDNSDIKVVP, GEIYKRWIILGLNKI, 5 HSNWRAMASDFNLPP, IKQFINMWQEVGKAMY, KRWIILGLNKIVRMY, KTAVQMAVFIHNFKR, KVYLAWVPAHKGIGG, QGQMVHQAI SPRTL N, QHLLQLTVWGIKQLQ, QKQITKIQNFRVYYR, SPAIFQSSMTKILEP, WAGIKQEF GIPYNPQ, WEFVNT PPLVKLWYQ, and YRKILRQRKIDRLID. This invention also provides the use of such an expression vector 10 for inducing an immune response *in vivo* in a mammalian subject as well as for preparation of a medicament for such use.

The invention therefore provides expression vectors encoding two or more HTL epitopes fused to a MHC class II targeting sequence, as well as expression vectors encoding a CTL epitope and a universal HTL epitope fused to an MHC class I targeting 15 sequence. The HTL epitope can be a universal HTL epitope (also referred to as a universal MHC class II epitope). The invention also provides expression vectors encoding two or more HTL epitopes fused to a MHC class II targeting sequence and encoding one or more CTL epitopes. The invention additionally provides methods of stimulating an immune response by administering an expression vector of the invention *in* 20 *vivo*, as well as methods of assaying the human immunogenicity of a human T cell peptide epitope *in vivo* in a non-human mammal.

In one aspect, the present invention provides an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding two or more heterologous peptide epitopes, wherein the heterologous peptide epitopes comprise two HTL peptide epitopes or a CTL peptide epitope and a universal HTL peptide epitope.

In another aspect, the present invention provides a method of inducing an immune response *in vivo* comprising administering to a mammalian subject an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding two or more heterologous peptide epitopes, wherein the heterologous peptide epitopes comprise two HTL peptide epitopes or a CTL peptide epitope and a universal HTL peptide epitope.

In another aspect, the present invention provides a method of inducing an immune response *in vivo* comprising administering to a mammalian subject an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding a heterologous human HTL peptide epitope.

In another aspect, the present invention provides a method of assaying the human immunogenicity of a human T cell peptide epitope *in vivo* in a non-human mammal, comprising the step of administering to the non-human mammal an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a heterologous human CTL or HTL peptide epitope.

In one embodiment, the heterologous peptide epitopes comprise two or more heterologous HTL peptide epitopes. In another embodiment, the heterologous peptide epitopes comprise a CTL peptide epitope and a universal HTL peptide epitope. In another embodiment, the heterologous peptide epitopes further comprise one to two or more heterologous CTL peptide epitopes. In another embodiment, the expression vector comprises both HTL and CTL peptide epitopes.

In one embodiment, one of the HTL peptide epitopes is a universal HTL epitope. In another embodiment, the universal HTL epitope is a pan DR epitope. In another embodiment, the pan DR epitope has the sequence AlaLysPheValAlaAlaTrpThrLeuLysAlaAlaAla (SEQ ID NO:38).

In one embodiment, the peptide epitopes are hepatitis B virus epitopes, hepatitis C virus epitopes, human immunodeficiency virus epitopes, human papilloma virus epitopes, MAGE epitopes, PSA epitopes, PSM epitopes, PAP epitopes, p53 epitopes, CEA epitopes, Her2/neu epitopes, or *Plasmodium* epitopes. In another
 5 embodiment, the peptide epitopes each have a sequence selected from the group consisting of the peptides depicted in Tables 1-8. In another embodiment, at least one of the peptide epitopes is an analog of a peptide depicted in Tables 1-8.

In one embodiment, the MHC targeting sequence comprises a region of a polypeptide selected from the group consisting of the Ii protein, LAMP-I, HLS-DM,
 10 HLA-DO, H2-DO, influenza matrix protein, hepatitis B surface antigen, hepatitis B virus core antigen, Ty particle, Ig- α protein, Ig- β protein, and Ig kappa chain signal sequence.

In one embodiment, the expression vector further comprises a second promoter sequence operably linked to a third nucleotide sequence encoding one or more heterologous HTL or CTL peptide epitopes. In another embodiment, the CTL peptide
 15 epitope comprises a structural motif for an HLA supertype, whereby the peptide CTL epitope binds to two or more members of the supertype with an affinity of greater than 500 nM. In another embodiment, the CTL peptide epitopes have structural motifs that provide binding affinity for more than one HLA allele supertype.

In one embodiment, the non-human mammal is a transgenic mouse that
 20 expresses a human HLA allele. In another embodiment, the human HLA allele is selected from the group consisting of A11 and A2.1. In another embodiment, the non-human mammal is a macaque that expresses a human HLA allele.

BRIEF DESCRIPTION OF THE DRAWINGS

25 Figure 1 shows the nucleotide and amino acid sequences (SEQ ID NOS:1 and 2, respectively) of the IiPADRE construct encoding a fusion of the murine Ii gene with a pan DR epitope sequence substituted for the CLIP sequence of the Ii protein.

Figure 2 shows the nucleotide and amino acid sequences (SEQ ID NOS:3 and 4, respectively) of the I80T construct encoding a fusion of the cytoplasmic domain,
 30 the transmembrane domain and part of the luminal domain of the Ii protein fused to multiple MHC class II epitopes.

Figure 3 shows the nucleotide and amino acid sequences (SEQ ID NOS:5 and 6, respectively) of the IiThfull construct encoding a fusion of the cytoplasmic domain, transmembrane domain and a portion of the luminal domain of the Ii protein

fused to multiple T helper epitopes and amino acid residues 101 to 215 of the Ii protein, which encodes the trimerization region of the Ii protein.

Figure 4 shows the nucleotide and amino acid sequences (SEQ ID NOS:7 and 8, respectively) of the KappaLAMP-Th construct encoding a fusion of the murine immunoglobulin kappa signal sequence fused to multiple T helper epitopes and the transmembrane and cytoplasmic domains of LAMP-1.

Figure 5 shows the nucleotide and amino acid sequences (SEQ ID NOS:9 and 10, respectively) of the H2M-Th construct encoding a fusion of the signal sequence of H2-M fused to multiple MHC class II epitopes and the transmembrane and cytoplasmic domains of H2-M.

Figure 6 shows the nucleotide and amino acid sequences (SEQ ID NOS:11 and 12, respectively) of the H2O-Th construct encoding a fusion of the signal sequence of H2-DO fused to multiple MHC class II epitopes and the transmembrane and cytoplasmic domains of H2-DO.

Figure 7 shows the nucleotide and amino acid sequences (SEQ ID NOS:13 and 14, respectively) of the PADRE-Influenza matrix construct encoding a fusion of a pan DR epitope sequence fused to the amino-terminus of influenza matrix protein.

Figure 8 shows the nucleotide and amino acid sequences (SEQ ID NOS:15 and 16, respectively) of the PADRE-HBV-s construct encoding a fusion of a pan DR epitope sequence fused to the amino-terminus of hepatitis B virus surface antigen.

Figure 9 shows the nucleotide and amino acid sequences (SEQ ID NOS:17 and 18, respectively) of the Ig-alphaTh construct encoding a fusion of the signal sequence of the Ig- α protein fused to multiple MHC class II epitopes and the transmembrane and cytoplasmic domains of the Ig- α protein.

Figure 10 shows the nucleotide and amino acid sequences (SEQ ID NOS:19 and 20, respectively) of the Ig-betaTh construct encoding a fusion of the signal sequence of the Ig- β protein fused to multiple MHC class II epitopes and the transmembrane and cytoplasmic domains of the Ig- β protein.

Figure 11 shows the nucleotide and amino acid sequences (SEQ ID NOS:21 and 22, respectively) of the SigTh construct encoding a fusion of the signal sequence of the kappa immunoglobulin fused to multiple MHC class II epitopes.

Figure 12 shows the nucleotide and amino acid sequences (SEQ ID NOS:23 and 24, respectively) of human HLA-DR, the invariant chain (Ii) protein.

Figure 13 shows the nucleotide and amino acid sequences (SEQ ID NOS:25 and 26, respectively) of human lysosomal membrane glycoprotein-1 (LAMP-1).

Figure 14 shows the nucleotide and amino acid sequences (SEQ ID NOS:27 and 28, respectively) of human HLA-DMB.

5 Figure 15 shows the nucleotide and amino acid sequences (SEQ ID NOS:29 and 30, respectively) of human HLA-DO beta.

Figure 16 shows the nucleotide and amino acid sequences (SEQ ID NOS:31 and 32, respectively) of the human MB-1 Ig- α .

10 Figure 17 shows the nucleotide and amino acid sequences (SEQ ID NOS:33 and 34, respectively) of human Ig- β protein.

Figure 18 shows a schematic diagram depicting the method of generating some of the constructs encoding a MHC class II targeting sequence fused to multiple MHC class II epitopes.

15 Figure 19 shows the nucleotide sequence of the vector pEP2 (SEQ ID NO:35).

Figure 20 shows the nucleotide sequence of the vector pMIN.0 (SEQ ID NO:36).

Figure 21 shows the nucleotide sequence of the vector pMIN.1 (SEQ ID NO:37).

20 Figure 22. Representative CTL responses in HLA-A2.1/K^b-H-2^{bxs} mice immunized with pMin.1 DNA. Splenocytes from primed animals were cultured in triplicate flasks and stimulated twice *in vitro* with each peptide epitope. Cytotoxicity of each culture was assayed in a ⁵¹Cr release assay against Jurkat-A2.1/K^b target cells in the presence (filled symbols, solid lines) or absence (open symbols, dotted lines) of peptide.
25 Each symbol represents the response of a single culture.

Figure 23. Presentation of viral epitopes to specific CTLs by Jurkat-A2.1/K^b tumor cells transfected with DNA minigene. Two constructs were used for transfection, pMin.1 and pMin.2-GFP. pMin.2-GFP-transfected targets cells were sorted by FACS and the population used in this experiment contained 60% fluorescent cells.
30 CTL stimulation was measured by quantitating the amount of IFN- γ release (A, B) or by lysis of ⁵¹Cr-labeled target cells (C, D, hatched bars). CTLs were stimulated with transfected cells (A, C) or with parental Jurkat-A2.1/K^b cells in the presence of 1 μ g/ml peptide (B, D). Levels of IFN- γ release and cytotoxicity for the different CTL lines in the absence of epitope ranged from 72-126 pg/ml and 2-6% respectively.

Figure 24. Summary of modified minigene constructs used to address variables critical for *in vivo* immunogenicity. The following modifications were incorporated into the prototype pMin.1 construct; A, deletion of PADRE HTL epitope; B, incorporation of the native HBV Pol 551 epitope that contains an alanine in position 9; C, deletion of the Ig kappa signal sequence; and D, switching position of the HBV Env 335 and HBV Pol 455 epitopes.

Figure 25. Examination of variables that may influence pMin.1 immunogenicity. *In vivo* CTL-inducing activity of pMin.1 is compared to modified constructs. For ease of comparison, the CTL response induced by each of the modified DNA minigene constructs (shaded bars) is compared separately in each of the four panels to the response induced by the prototype pMin.1 construct (solid bars). The geometric mean response of CTL-positive cultures from two to five independent experiments are shown. Numbers shown with each bar indicate the number of positive cultures/total number tested for that particular epitope. The ratio of positive cultures/total tested for the pMin.1 group is shown in panel A and is the same for the remaining Figure panels (see Example V, Materials and Methods, *in vitro* CTL cultures, for the definition of a positive CTL culture). Theradigm responses were obtained by immunizing animals with the lipopeptide and stimulating and testing splenocyte cultures with the HBV Core 18-27 peptide.

20

DEFINITIONS

An "HTL" peptide epitope or an "MHC II epitope" is an MHC class II restricted epitope, i.e., one that is bound by an MHC class II molecule.

25 A "CTL" peptide epitope or an "MHC I epitope" is an MHC class I restricted epitope, i.e., one that is bound by an MHC class I molecule.

An "MHC targeting sequence" refers to a peptide sequence that targets a polypeptide, e.g., comprising a peptide epitope, to a cytosolic pathway (e.g., an MHC class I antigen processing pathway), an endoplasmic reticulum pathway, or an endocytic pathway (e.g., an MHC class II antigen processing pathway).

30 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 polypeptide comprising subsequence from different polypeptides, peptide epitopes from the same polypeptide that are not naturally in an adjacent position, or repeats of a single peptide epitope.

As used herein, the term "universal MHC class II epitope" or a "universal HTL epitope" refers to a MHC class II peptide epitope that binds to gene products of multiple MHC class II alleles. For example, the DR, DP and DQ alleles are human MHC II alleles. Generally, a unique set of peptides binds to a particular gene product of a MHC class II allele. In contrast, a universal MHC class II epitope is able to bind to gene products of multiple MHC class II alleles. A universal MHC class II epitope binds to 2 or more MHC class II alleles, generally 3 or more MHC class II alleles, and particularly 5 or more MHC class II alleles. Thus, the presence of a universal MHC class II epitope in an expression vector is advantageous in that it functions to increase the number of allelic MHC class II molecules that can bind to the peptide and, consequently, the number of Helper T lymphocytes that are activated.

Universal MHC class II epitopes are well known in the art and include, for example, epitopes such as the "pan DR epitopes," also referred to as "PADRE" (Alexander *et al.*, *Immunity* 1:751-761 (1994); WO 95/07707, WO 98/32456 and WO 99/61916). A "pan DR binding peptide" or a "PADRE" peptide of

the invention is a peptide capable of binding at least about 7 different DR molecules, preferably 7 of the 12 most common DR molecules, most preferably 9 of the 12 most common DR molecules (DR1, 2w2b, 2w2a, 3, 4w4, 4w14, 5, 7, 52a, 52b, 52c, and 53), or alternatively, 50% of a panel of DR molecules representative of greater than or equal to 75% of the human population, preferably greater than or equal to 80% of the human population. Pan DR epitopes can bind to a number of DR alleles and are strongly immunogenic for T cells. For example, pan DR epitopes were found to be more effective at inducing an immune response than natural MHC class II epitopes (Alexander, *supra*). An example of a PADRE epitope is the peptide AlaLysPheValAlaAlaTrpThrLeuLysAlaAlaAla (SEQ ID NO:38).

With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably. It is to be appreciated, however, that isolated or purified protein or peptide molecules larger than and comprising an epitope of the invention are still within the bounds of the invention.

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC₅₀ (or K_D) of less than 50 nM. "Intermediate affinity" is binding with an IC₅₀ (or K_D) of between about 50 and about 500 nM. "High affinity" with respect to binding to HLA class II molecules is defined as binding with an K_D of less than 100 nM. "Intermediate affinity" is binding with a K_D of between about 100 and about 1000 nM. Assays for determining binding are described in detail, e.g., in PCT publications WO 94/20127 and WO 94/03205. Alternatively, binding is expressed relative to a reference peptide. As a particular assay becomes more, or less, sensitive, the IC₅₀s of the peptides tested may change somewhat. However, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC₅₀ of the reference peptide increases 10-fold, the IC₅₀ values of the test peptides will also shift approximately 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC₅₀, relative to the IC₅₀ of a standard peptide.

Throughout this disclosure, results are expressed in terms of "IC₅₀s." IC₅₀ is the concentration of peptide in a binding assay at which 50% inhibition of binding of a reference peptide is observed. Given the conditions in which the assays are run (i.e., limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. It should be noted that IC₅₀ values can change, often dramatically, if the assay conditions are varied, and depending on the particular reagents used (e.g., HLA preparation, etc.). For example, excessive concentrations of HLA molecules will increase the apparent measured IC₅₀ of a given ligand.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or

have a specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithms using default program parameters or by manual alignment and visual inspection.

5 The phrases “isolated” or “biologically pure” refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their in situ environment.

10 “Major histocompatibility complex” or “MHC” is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, *see* Paul, *Fundamental Immunology* (3rd ed. 1993).

15 “Human leukocyte antigen” or “HLA” is a human class I or class II major histocompatibility complex (MHC) protein (*see, e.g.,* Stites, et al., *Immunology*, (8th ed., 1994).

 An “HLA supertype or family”, as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I
20 molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like supertype molecules (where xx denotes a particular HLA type), are synonyms.

 The term “motif” refers to the pattern of residues in a peptide of defined
25 length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

30 A “supermotif” is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Thus, a preferably is recognized with high or intermediate affinity (as defined herein) by two or more HLA antigens.

 “Cross-reactive binding” indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing oligopeptides of the invention are 13
5 residues or less in length and usually consist of between about 8 and about 11 residues, preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

An "immunogenic peptide" or "peptide epitope" is a peptide which
10 comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

15 A "protective immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

20 The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

"Synthetic peptide" refers to a peptide that is not naturally occurring, but is man-made using such methods as chemical synthesis or recombinant DNA technology.

The nomenclature used to describe peptide compounds follows the
25 conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the
30 formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single

letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G.

As used herein, the term "expression vector" is intended to refer to a
 5 nucleic acid molecule capable of expressing an antigen of interest such as a MHC class I or class II epitope in an appropriate target cell. An expression vector can be, for example, a plasmid or virus, including DNA or RNA viruses. The expression vector contains such a promoter element to express an antigen of interest in the appropriate cell or tissue in order to stimulate a desired immune response.

10

DETAILED DESCRIPTION OF THE INVENTION

Cytotoxic T lymphocytes (CTLs) and helper T lymphocytes (HTLs) are critical for immunity against infectious pathogens; such as viruses, bacteria, and protozoa; tumor cells; autoimmune diseases and the like. The present invention provides
 15 minigenes that encode peptide epitopes which induce a CTL and/or HTL response. The minigenes of the invention also include an MHC targeting sequence. A variety of minigenes encoding different epitopes can be tested for immunogenicity using an HLA transgenic mouse. The epitopes are typically a combination of at least two or more HTL epitopes, or a CTL epitope plus a universal HTL epitope, and optionally include additional
 20 HTL and/or CTL epitopes. Two, three, four, five, six, seven, eight, nine, ten, twenty, thirty, forty or about fifty different epitopes, either HTL and/or CTL, can be included in the minigene, along with the MHC targeting sequence. The epitopes can have different HLA restriction. Epitopes to be tested include those derived from viruses such as HIV, HBV, HCV, HSV, CMV, HPV, and HTLV; cancer antigens such as p53, Her2/Neu,
 25 MAGE, PSA, human papilloma virus, and CEA; parasites such as *Trypanosoma*, *Plasmodium*, *Leishmania*, *Giardia*, *Entamoeba*; autoimmune diseases such as rheumatoid arthritis, myasthenia gravis, and lupus erythematosus; fungi such as *Aspergillus* and *Candida*; and bacteria such as *Escherichia coli*, *Staphylococci*, *Chlamydia*, *Mycobacteria*, *Streptococci*, and *Pseudomonas*. The epitopes to be encoded by the minigene are selected
 30 and tested using the methods described in published PCT applications WO 93/07421, WO 94/02353, WO 95/01000, WO 97/04451, and WO 97/05348.

HTL and CTL Epitopes

The expression vectors of the invention encode one or more MHC class II and/or class I epitopes and an MHC targeting sequence. Multiple MHC class II or class I epitopes present in an expression vector can be derived from the same antigen, or the MHC epitopes can be derived from different antigens. For example, an expression vector can contain one or more MHC epitopes that can be derived from two different antigens of the same virus or from two different antigens of different viruses. Furthermore, any MHC epitope can be used in the expression vectors of the invention. For example, any single MHC epitope or a combination of the MHC epitopes shown in Tables 1 to 8 can be used in the expression vectors of the invention. Other peptide epitopes can be selected by one of skill in the art, e.g., by using a computer to select epitopes that contain HLA allele-specific motifs or supermotifs. The expression vectors of the invention can also encode one or more universal MHC class II epitopes, e.g., PADRE (*see, e.g.*, SEQ ID NO:38).

Universal MHC class II epitopes can be advantageously combined with other MHC class I and class II epitopes to increase the number of cells that are activated in response to a given antigen and provide broader population coverage of MHC-reactive alleles. Thus, the expression vectors of the invention can encode MHC epitopes specific for an antigen, universal MHC class II epitopes, or a combination of specific MHC epitopes and at least one universal MHC class II epitope.

MHC class I epitopes are generally about 5 to 15 amino acids in length, in particular about 8 to 11 amino acids in length. MHC class II epitopes are generally about 10 to 25 amino acids in length, in particular about 13 to 21 amino acids in length. A MHC class I or II epitope can be derived from any desired antigen of interest. The antigen of interest can be a viral antigen, surface receptor, tumor antigen, oncogene, enzyme, or any pathogen, cell or molecule for which an immune response is desired. Epitopes can be selected based on their ability to bind one or multiple HLA alleles, and can also be selected using the "analog" technique described below.

Targeting Sequences

The expression vectors of the invention encode one or more MHC epitopes operably linked to a MHC targeting sequence. The use of a MHC targeting sequence enhances the immune response to an antigen, relative to delivery of antigen alone, by

directing the peptide epitope to the site of MHC molecule assembly and transport to the cell surface, thereby providing an increased number of MHC molecule-peptide epitope complexes available for binding to and activation of T cells.

MHC class I targeting sequences are used in the present invention, e.g., those sequences that target an MHC class I epitope peptide to a cytosolic pathway or to the endoplasmic reticulum (see, e.g., Rammensee *et al.*, *Immunogenetics* 41:178-228 (1995)). For example, the cytosolic pathway processes endogenous antigens that are expressed inside the cell. Although not wishing to be bound by any particular theory, cytosolic proteins are thought to be at least partially degraded by an endopeptidase activity of a proteasome and then transported to the endoplasmic reticulum by the TAP molecule (transporter associated with processing). In the endoplasmic reticulum, the antigen binds to MHC class I molecules. Endoplasmic reticulum signal sequences bypass the cytosolic processing pathway and directly target endogenous antigens to the endoplasmic reticulum, where proteolytic degradation into peptide fragments occurs. Such MHC class I targeting sequences are well known in the art, and include, e.g., signal sequences such as those from Ig kappa, tissue plasminogen activator or insulin. A preferred signal peptide is the human Ig kappa chain sequence. Endoplasmic reticulum signal sequences can also be used to target MHC class II epitopes to the endoplasmic reticulum, the site of MHC class I molecule assembly.

MHC class II targeting sequences are also used in the invention, e.g., those that target a peptide to the endocytic pathway. These targeting sequences typically direct extracellular antigens to enter the endocytic pathway, which results in the antigen being transferred to the lysosomal compartment where the antigen is proteolytically cleaved into antigen peptides for binding to MHC class II molecules. As with the normal processing of exogenous antigen, a sequence that directs a MHC class II epitope to the endosomes of the endocytic pathway and/or subsequently to lysosomes, where the MHC class II epitope can bind to a MHC class II molecule, is a MHC class II targeting sequence. For example, group of MHC class II targeting sequences useful in the invention are lysosomal targeting sequences, which localize polypeptides to lysosomes. Since MHC class II molecules typically bind to antigen peptides derived from proteolytic processing of endocytosed antigens in lysosomes, a lysosomal targeting sequence can function as a MHC class II targeting sequence. Lysosomal targeting sequences are well known in the art and include sequences found in the lysosomal proteins LAMP-1 and

LAMP-2 as described by August *et al.* (U.S. Patent No. 5,633,234, issued May 27, 1997).

Other lysosomal proteins that contain lysosomal targeting sequences include HLA-DM. HLA-DM is an endosomal/lysosomal protein that functions in
 5 facilitating binding of antigen peptides to MHC class II molecules. Since it is located in the lysosome, HLA-DM has a lysosomal targeting sequence that can function as a MHC class II molecule targeting sequence (Copier *et al.*, *J. Immunol.* 157:1017-1027 (1996)).

The resident lysosomal protein HLA-DO can also function as a lysosomal
 10 targeting sequence. In contrast to the above described resident lysosomal proteins LAMP-1 and HLA-DM, which encode specific Tyr-containing motifs that target proteins to lysosomes, HLA-DO is targeted to lysosomes by association with HLA-DM (Liljedahl *et al.*, *EMBO J.* 15:4817-4824 (1996)), which is incorporated herein by reference. Therefore, the sequences of HLA-DO that cause association with HLA-DM and,
 15 consequently, translocation of HLA-DO to lysosomes can be used as MHC class II targeting sequences. Similarly, the murine homolog of HLA-DO, H2-DO, can be used to derive a MHC class II targeting sequence. A MHC class II epitope can be fused to HLA-DO or H2-DO and targeted to lysosomes.

In another example, the cytoplasmic domains of B cell receptor subunits
 20 Ig- α and Ig- β mediate antigen internalization and increase the efficiency of antigen presentation (Bonnerot *et al.*, *Immunity* 3:335-347 (1995)).

Therefore, the cytoplasmic domains of the Ig- α and Ig- β proteins can function as MHC class II targeting sequences that target a MHC class II epitope to the endocytic pathway for processing and binding to MHC class II molecules.

25 Another example of a MHC class II targeting sequence that directs MHC class II epitopes to the endocytic pathway is a sequence that directs polypeptides to be secreted, where the polypeptide can enter the endosomal pathway. These MHC class II targeting sequences that direct polypeptides to be secreted mimic the normal pathway by which exogenous, extracellular antigens are processed into peptides that bind to MHC
 30 class II molecules. Any signal sequence that functions to direct a polypeptide through the endoplasmic reticulum and ultimately to be secreted can function as a MHC class II targeting sequence so long as the secreted polypeptide can enter the endosomal/lysosomal pathway and be cleaved into peptides that can bind to MHC class II molecules. An

example of such a fusion is shown in Figure 11, where the signal sequence of kappa immunoglobulin is fused to multiple MHC class II epitopes.

In another example, the Ii protein binds to MHC class II molecules in the endoplasmic reticulum, where it functions to prevent peptides present in the endoplasmic reticulum from binding to the MHC class II molecules. Therefore, fusion of a MHC class II epitope to the Ii protein targets the MHC class II epitope to the endoplasmic reticulum and a MHC class II molecule. For example, the CLIP sequence of the Ii protein can be removed and replaced with a MHC class II epitope sequence so that the MHC class II epitope is directed to the endoplasmic reticulum, where the epitope binds to a MHC class II molecule.

In some cases, antigens themselves can serve as MHC class II or I targeting sequences and can be fused to a universal MHC class II epitope to stimulate an immune response. Although cytoplasmic viral antigens are generally processed and presented as complexes with MHC class I molecules, long-lived cytoplasmic proteins such as the influenza matrix protein can enter the MHC class II molecule processing pathway (Guéguen & Long, *Proc. Natl. Acad. Sci. USA* 93:14692-14697 (1996)).

Therefore, long-lived cytoplasmic proteins can function as a MHC class II targeting sequence. For example, an expression vector encoding influenza matrix protein fused to a universal MHC class II epitope can be advantageously used to target influenza antigen and the universal MHC class II epitope to the MHC class II pathway for stimulating an immune response to influenza.

Other examples of antigens functioning as MHC class II targeting sequences include polypeptides that spontaneously form particles. The polypeptides are secreted from the cell that produces them and spontaneously form particles, which are taken up into an antigen-presenting cell by endocytosis such as receptor-mediated endocytosis or are engulfed by phagocytosis. The particles are proteolytically cleaved into antigen peptides after entering the endosomal/lysosomal pathway.

One such polypeptide that spontaneously forms particles is HBV surface antigen (HBV-S) (Diminsky *et al.*, *Vaccine* 15:637-647 (1997); Le Borgne *et al.*, *Virology* 240:304-315 (1998)).

Another polypeptide that spontaneously forms particles is HBV core antigen (Kuhröber *et al.*, *International Immunol.* 9:1203-1212 (1997)).

Still another polypeptide that spontaneously forms particles is the yeast Ty protein (Weber *et al.*, *Vaccine* 13:831-834 (1995)).

For example, an expression vector containing HBV-S antigen fused to a universal MHC class II epitope can be advantageously used to target HBV-S antigen and the universal MHC class II epitope to the MHC class II pathway for stimulating an immune response to HBV.

5

Binding Affinity of Peptide Epitopes for HLA Molecules

The large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have a binding affinity for class I HLA molecules of less than 500 nM. HTL-inducing peptides preferably include those that have a binding affinity for class II HLA molecules of less than 1000 nM. For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in vaccines or in cellular screening analyses.

Higher HLA binding affinity is typically correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. In accordance with these principles, close to 90% of high binding peptides have been found to be immunogenic, as contrasted with about 50% of the peptides which bind with intermediate affinity. Moreover, higher binding affinity peptides leads to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high affinity binding peptide is used. Thus, in preferred embodiments of the invention, high binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (Sette *et al.*,
 5 *J. Immunol.* 153:5586-5592 (1994)). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL (peripheral blood
 10 lymphocytes) from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity threshold of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant
 15 selection in the shaping of T cell responses (*see, e.g., Schaeffer et al. Proc. Natl. Acad. Sci. USA* 86:4649-4653, 1989).

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g., Southwood et al. J. Immunology* 160:3363-3373 (1998), and WO 99/61916). In order to
 20 define a biologically significant threshold of DR binding affinity, a database of the binding affinities of 32 DR-restricted epitopes for their restricting element (i.e., the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, i.e. binding affinities of less than 100 nM. In the other half of the cases (16 of 32), DR restriction was
 25 associated with intermediate affinity (binding affinities in the 100-1000 nM range). In only one of 32 cases was DR restriction associated with an IC50 of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

30 Peptide Epitope Binding Motifs and Supermotifs

In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets.

For HLA molecule pocket analyses, the residues comprising the B and F pockets of HLA class I molecules as described in crystallographic studies were analyzed (Guo *et al.*, *Nature* 360:364 (1992); Saper *et al.*, *J. Mol. Biol.* 219:277 (1991); Madden *et al.*, *Cell* 75:693 (1993); Parham *et al.*, *Immunol. Rev.* 143:141 (1995)). In these analyses, residues 9, 45, 63, 66, 67, 70, and 99 were considered to make up the B pocket; and the B pocket was deemed to determine the specificity for the amino acid residue in the second position of peptide ligands. Similarly, residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket; the F pocket was deemed to determine the specificity for the C-terminal residue of a peptide ligand bound by the HLA class I molecule.

Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912 (1994)) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecules with high or intermediate affinity. Of these 22 peptides, 20, (i.e., 91%), were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques eliminates screening of 90% of the potential epitopes in a target antigen protein sequence.

Peptides of the present invention may also include epitopes that bind to MHC class II DR molecules. There is a significant difference between class I and class II HLA molecules. This difference corresponds to the fact that, although a stringent size restriction and motif position relative to the binding pocket exists for peptides that bind to class I molecules, a greater degree of heterogeneity in both size and binding frame

position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands.

This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the residues occupying position 1 and position 6 of peptides complexed with DRB*0101 engage two complementary pockets on the DRBa*0101 molecules, with the P1 position corresponding to the most crucial anchor residue and the deepest hydrophobic pocket (*see, e.g., Madden, Ann. Rev. Immunol.* 13:587 (1995)). Other studies have also pointed to the P6 position as a crucial anchor residue for binding to various other DR molecules.

Thus, peptides of the present invention are identified by any one of several HLA class I or II -specific amino acid motifs (*see, e.g., Tables I-III of WO 02/019986 and U.S. 6,689,363*). If the presence of the motif corresponds to the ability to bind several allele-specific HLA antigens it is referred to as a supermotif. The allele-specific HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA "supertype."

Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few "immunodominant" determinants (Zinkernagel *et al., Adv. Immunol.* 27:5159 (1979); Bennink *et al., J. Exp. Med.* 168:1935-1939 (1988); Rawle *et al., J. Immunol.* 146:3977-3984 (1991)). It has been recognized that immunodominance (Benacerraf *et al., Science* 175:273-279 (1972)) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello *et al., J. Immunol.* 131:1635 (1983)); Rosenthal *et al., Nature* 267:156-158 (1977)), or being selectively recognized by the existing TCR (T cell receptor) specificity (repertoire theory) (Klein, *Immunology, The Science of Self on self Discrimination*, pp. 270-310 (1982)). It has been demonstrated that additional factors, mostly linked to processing events, can also play a key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz *et al., Annu. Rev. Immunol.* 11:729-766 (1993)).

The concept of dominance and subdominance is relevant to immunotherapy of both infectious diseases and cancer. For example, in the course of chronic viral disease, recruitment of subdominant epitopes can be important for successful clearance of the infection, especially if dominant CTL or HTL specificities have been inactivated by functional tolerance, suppression, mutation of viruses and other mechanisms (Franco *et al.*, *Curr. Opin. Immunol.* 7:524-531 (1995)). In the case of cancer and tumor antigens, CTLs recognizing at least some of the highest binding affinity peptides might be functionally inactivated. Lower binding affinity peptides are preferentially recognized at these times, and may therefore be preferred in therapeutic or prophylactic anti-cancer vaccines.

In particular, it has been noted that a significant number of epitopes derived from known non-viral tumor associated antigens (TAA) bind HLA class I with intermediate affinity (IC₅₀ in the 50-500 nM range). For example, it has been found that 8 of 15 known TAA peptides recognized by tumor infiltrating lymphocytes (TIL) or CTL bound in the 50-500 nM range. (These data are in contrast with estimates that 90% of known viral antigens were bound by HLA class I molecules with IC₅₀ of 50 nM or less, while only approximately 10% bound in the 50-500 nM range (Sette *et al.*, *J. Immunol.*, 153:558-5592 (1994)). In the cancer setting this phenomenon is probably due to elimination, or functional inhibition of the CTL recognizing several of the highest binding peptides, presumably because of T cell tolerization events.

Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow extant T cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response. This ability would greatly enhance the usefulness of peptide-based vaccines and therapeutic agents.

Thus, although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to further increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other properties of the peptides such as binding affinity or peptide stability.

Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (i.e., analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the
 5 broadest cross-reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in WO 02/019986 and U.S. 6,689,363.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate with binding to certain HLA class I and II molecules. The motifs or supermotifs
 10 are defined by having primary anchors, and in many cases secondary anchors (see Tables I-III of WO 02/019986 and U.S. 6,689,363). Analog peptides can be created by substituting amino acids residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and motifs that
 15 have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively, of WO 02/019986 and U.S. 6,689,363.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind to the respective motif or supermotif
 20 (see Tables II and II of WO 02/019986 and U.S. 6,689,363). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the methods described therein. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of analyzed peptides, the incidence of cross-reactivity increases from 22% to 37% (I., Sidney *et al.*, *Hu. Immunol.* 45:79
 25 (1996)). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues
 30 associated with high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, a failure to elicit helper T cells that cross-react with the wild type peptides), the analog peptide may

be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. In both class I and class II systems it will be desirable to use as targets, cells that have been either infected or transfected with the appropriate genes to
 5 establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I peptides exhibiting binding affinities of 500-50000 nM, and carrying an acceptable but
 10 suboptimal primary anchor residue at one or both positions can be "fixed" by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in,
 15 e.g., a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine (C) can be substituted out in favor of gamma-amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting gamma-amino butyric acid for C not only alleviates this problem, but
 20 actually improves binding and crossbinding capability in certain instances (Sette *et al*, *In: Persistent Viral Infections* (Ahmed & Chen, eds., 1998)). Substitution of cysteine with gamma-amino butyric acid may occur at any residue of a peptide epitope, i.e., at either anchor or non-anchor positions.

25 Expression Vectors and Construction of a Minigene

The expression vectors of the invention contain at least one promoter element that is capable of expressing a transcription unit encoding the antigen of interest, for example, a MHC class I epitope or a MHC class II epitope and an MHC targeting sequence in the appropriate cells of an organism so that the antigen is expressed and
 30 targeted to the appropriate MHC molecule. For example, if the expression vector is administered to a mammal such as a human, a promoter element that functions in a human cell is incorporated into the expression vector. An example of an expression vector useful for expressing the MHC class II epitopes fused to MHC class II targeting

sequences and the MHC class I epitopes described herein is the pEP2 vector described in Example IV.

This invention relies on routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include
 5 Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994); *Oligonucleotide Synthesis: A Practical Approach* (Gait, ed., 1984); Kuijpers, *Nucleic Acids Research* 18(17):5197 (1994); Dueholm, *J. Org. Chem.* 59:5767-5773 (1994); *Methods in Molecular Biology*, volume
 10 20 (Agrawal, ed.); and Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes*, e.g., Part I, chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" (1993)).

The minigenes are comprised of two or many different epitopes (*see, e.g.*, Tables 1-8). The nucleic acid encoding the epitopes are assembled in a minigene
 15 according to standard techniques. In general, the nucleic acid sequences encoding minigene epitopes are isolated using amplification techniques with oligonucleotide primers, or are chemically synthesized. Recombinant cloning techniques can also be used when appropriate. Oligonucleotide sequences are selected which either amplify (when using PCR to assemble the minigene) or encode (when using synthetic oligonucleotides to
 20 assemble the minigene) the desired epitopes.

Amplification techniques using primers are typically used to amplify and isolate sequences encoding the epitopes of choice from DNA or RNA (*see U.S. Patents* 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Methods such as polymerase chain reaction (PCR) and ligase chain
 25 reaction (LCR) can be used to amplify epitope nucleic acid sequences directly from mRNA, from cDNA, from genomic libraries or cDNA libraries. Restriction endonuclease sites can be incorporated into the primers. Minigenes amplified by the PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

Synthetic oligonucleotides can also be used to construct minigenes. This
 30 method is performed using a series of overlapping oligonucleotides, representing both the sense and non-sense strands of the gene. These DNA fragments are then annealed, ligated and cloned. Oligonucleotides that are not commercially available can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, *Tetrahedron Letts.* 22:1859-1862 (1981), using an

automated synthesizer, as described in Van Devanter *et al.*, *Nucleic Acids Res.* 12:6159-6168 (1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom.* 255:137-149 (1983).

5 The epitopes of the minigene are typically subcloned into an expression vector that contains a strong promoter to direct transcription, as well as other regulatory sequences such as enhancers and polyadenylation sites. Suitable promoters are well known in the art and described, e.g., in Sambrook *et al.* and Ausubel *et al.* Eukaryotic expression systems for mammalian cells are well known in the art and are commercially
10 available. Such promoter elements include, for example, cytomegalovirus (CMV), Rous sarcoma virus LTR and SV40.

 The expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the minigene in host cells. A typical expression cassette thus contains a promoter operably
15 linked to the minigene and signals required for efficient polyadenylation of the transcript. Additional elements of the cassette may include enhancers and introns with functional splice donor and acceptor sites.

 In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide
20 for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

 The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic cells may be used. Expression vectors containing regulatory
25 elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein Bar virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 later promoter, metallothionein promoter,
30 murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells. In one embodiment, the vector pEP2 is used in the present invention.

 Other elements that are typically included in expression vectors also include a replicon that functions in *E. coli*, a gene encoding antibiotic resistance to permit

selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular antibiotic resistance gene chosen is not critical, any of the many resistance genes known in the art are suitable. The prokaryotic sequences are preferably chosen
 5 such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

Administration *In Vivo*

The invention also provides methods for stimulating an immune response
 10 by administering an expression vector of the invention to an individual. Administration of an expression vector of the invention for stimulating an immune response is advantageous because the expression vectors of the invention target MHC epitopes to MHC molecules, thus increasing the number of CTL and HTL activated by the antigens encoded by the expression vector.

15 Initially, the expression vectors of the invention are screened in mouse to determine the expression vectors having optimal activity in stimulating a desired immune response. Initial studies are therefore carried out, where possible, with mouse genes of the MHC targeting sequences. Methods of determining the activity of the expression vectors of the invention are well known in the art and include, for example, the uptake of
 20 ³H-thymidine to measure T cell activation and the release of ⁵¹Cr to measure CTL activity as described below in Examples II and III. Experiments similar to those described in Example IV are performed to determine the expression vectors having activity at stimulating an immune response. The expression vectors having activity are further tested in human. To circumvent potential adverse immunological responses to encoded
 25 mouse sequences, the expression vectors having activity are modified so that the MHC class II targeting sequences are derived from human genes. For example, substitution of the analogous regions of the human homologs of genes containing various MHC class II targeting sequences are substituted into the expression vectors of the invention. Examples of such human homologs of genes containing MHC class II targeting sequences are shown in Figures 12 to 17. Expression vectors containing human MHC class II
 30 targeting sequences, such as those described in Example I below, are tested for activity at stimulating an immune response in human.

The invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an expression vector of the invention.

Pharmaceutically acceptable carriers are well known in the art and include aqueous or non-aqueous solutions, suspensions and emulsions, including physiologically buffered saline, alcohol/aqueous solutions or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters.

5 A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize the expression vector or increase the absorption of the expression vector. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants such as ascorbic acid or glutathione, chelating agents, low molecular weight polypeptides, antimicrobial agents, inert gases or other stabilizers or excipients. Expression vectors can additionally be complexed with other components such as peptides, polypeptides and carbohydrates. Expression vectors can also be complexed to particles or beads that can be administered to an individual, for example, using a vaccine gun. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a
10 physiologically acceptable compound, depends, for example, on the route of
15 administration of the expression vector.

The invention further relates to methods of administering a pharmaceutical composition comprising an expression vector of the invention to stimulate an immune response. The expression vectors are administered by methods well known in the art as
20 described in Donnelly *et al.* (*Ann. Rev. Immunol.* 15:617-648 (1997)); Felgner *et al.* (U.S. Patent No. 5,580,859, issued December 3, 1996); Felgner (U.S. Patent No. 5,703,055, issued December 30, 1997); and Carson *et al.* (U.S. Patent No. 5,679,647, issued October 21, 1997). In one embodiment, the
minigene is administered as naked nucleic acid.

25 A pharmaceutical composition comprising an expression vector of the invention can be administered to stimulate an immune response in a subject by various routes including, for example, orally, intravaginally, rectally, or parenterally, such as intravenously, intramuscularly, subcutaneously, intraorbitally, intracapsularly, intraperitoneally, intracisternally or by passive or facilitated absorption through the skin
30 using, for example, a skin patch or transdermal iontophoresis, respectively. Furthermore, the composition can be administered by injection, intubation or topically, the latter of which can be passive, for example, by direct application of an ointment or powder, or active, for example, using a nasal spray or inhalant. An expression vector also can be administered as a topical spray, in which case one component of the composition is an

appropriate propellant. The pharmaceutical composition also can be incorporated, if desired, into liposomes, microspheres or other polymer matrices (Felgner *et al.*, U.S. Patent No. 5,703,055; Gregoriadis, *Liposome Technology*, Vols. I to III (2nd ed. 1993).

Liposomes, for example, which
5 consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The expression vectors of the invention can be delivered to the interstitial spaces of tissues of an animal body (Felgner *et al.*, U.S. Patent Nos. 5,580,859 and 5,703,055). Administration of expression vectors of the invention to muscle is a
10 particularly effective method of administration, including intradermal and subcutaneous injections and transdermal administration. Transdermal administration, such as by iontophoresis, is also an effective method to deliver expression vectors of the invention to muscle. Epidermal administration of expression vectors of the invention can also be employed. Epidermal administration involves mechanically or chemically irritating the
15 outermost layer of epidermis to stimulate an immune response to the irritant (Carson *et al.*, U.S. Patent No. 5,679,647).

Other effective methods of administering an expression vector of the invention to stimulate an immune response include mucosal administration (Carson *et al.*, U.S. Patent No. 5,679,647). For mucosal administration, the most effective method of
20 administration includes intranasal administration of an appropriate aerosol containing the expression vector and a pharmaceutical composition. Suppositories and topical preparations are also effective for delivery of expression vectors to mucosal tissues of genital, vaginal and ocular sites. Additionally, expression vectors can be complexed to particles and administered by a vaccine gun.

25 The dosage to be administered is dependent on the method of administration and will generally be between about 0.1 µg up to about 200 µg. For example, the dosage can be from about 0.05 µg/kg to about 50 mg/kg, in particular about 0.005-5 mg/kg. An effective dose can be determined, for example, by measuring the immune response after administration of an expression vector. For example, the
30 production of antibodies specific for the MHC class II epitopes or MHC class I epitopes encoded by the expression vector can be measured by methods well known in the art, including ELISA or other immunological assays. In addition, the activation of T helper cells or a CTL response can be measured by methods well known in the art including, for

example, the uptake of ^3H -thymidine to measure T cell activation and the release of ^{51}Cr to measure CTL activity (see Examples II and III below).

The pharmaceutical compositions comprising an expression vector of the invention can be administered to mammals, particularly humans, for prophylactic or
5 therapeutic purposes. Examples of diseases that can be treated or prevented using the expression vectors of the invention include infection with HBV, HCV, HIV and CMV as well as prostate cancer, renal carcinoma, cervical carcinoma, lymphoma, condyloma acuminatum and acquired immunodeficiency syndrome (AIDS).

In therapeutic applications, the expression vectors of the invention are
10 administered to an individual already suffering from cancer, autoimmune disease or infected with a virus. Those in the incubation phase or acute phase of the disease can be treated with expression vectors of the invention, including those expressing all universal MHC class II epitopes, separately or in conjunction with other treatments, as appropriate.

In therapeutic and prophylactic applications, pharmaceutical compositions
15 comprising expression vectors of the invention are administered to a patient in an amount sufficient to elicit an effective immune response to an antigen and to ameliorate the signs or symptoms of a disease. The amount of expression vector to administer that is sufficient to ameliorate the signs or symptoms of a disease is termed a therapeutically effective dose. The amount of expression vector sufficient to achieve a therapeutically
20 effective dose will depend on the pharmaceutical composition comprising an expression vector of the invention, the manner of administration, the state and severity of the disease being treated, the weight and general state of health of the patient and the judgment of the prescribing physician.

25

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
30 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 example is provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

5

EXAMPLE I: Construction of Expression Vectors Containing MHC Class II Epitopes

This example shows construction of expression vectors containing MHC class II epitopes that can be used to target antigens to MHC class II molecules.

Expression vectors comprising DNA constructs were prepared using
 10 overlapping oligonucleotides, polymerase chain reaction (PCR) and standard molecular biology techniques (Dieffenbach & Dveksler, *PCR Primer: A Laboratory Manual* (1995); Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed., 1989).

To generate full length wild type Ii, the full length invariant chain was
 15 amplified, cloned, and sequenced and used in the construction of the three invariant chain constructs. Except where noted, the source of cDNA for all the constructs listed below was Mouse Spleen Marathon-Ready cDNA made from Balb/c males (Clontech; Palo Alto CA). The primer pairs were the oligonucleotide
 GCTAGCGCCGCCACCATGGATGACCAACGCGACCTC (SEQ ID NO:40), which is
 20 designated murli-F and contains an NheI site followed by the consensus Kozak sequence and the 5' end of the Ii cDNA; and the oligonucleotide
 GGTACCTCACAGGGTGACTTGACCCAG (SEQ ID NO:41), which is designated murli-R and contains a KpnI site and the 3' end of the Ii coding sequence.

For the PCR reaction, 5 µl of spleen cDNA and 250 nM of each primer
 25 were combined in a 100 µl reaction with 0.25 mM each dNTP and 2.5 units of *Pfu* polymerase in *Pfu* polymerase buffer containing 10 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% TRITON X-100 and 100 µg/ml bovine serum albumin (BSA). A Perkin/Elmer 9600 PCR machine (Perkin Elmer; Foster City CA) was used and the cycling conditions were: 1 cycle of 95°C for 5 minutes, followed by 30
 30 cycles of 95°C for 15 seconds, 52°C for 30 seconds, and 72°C for 1 minute. The PCR reaction was run on a 1% agarose gel, and the 670 base pair product was cut out, purified by spinning through a Millipore Ultrafree-MC filter (Millipore; Bedford MA) and cloned into pCR-Blunt from Invitrogen (San Diego, CA). Individual clones were screened by

sequencing, and a correct clone (named bIi#3) was used as a template for the helper constructs.

DNA constructs containing pan DR epitope sequences and MHC II targeting sequences derived from the Ii protein were prepared. The Ii murine protein has
5 been previously described (Zhu & Jones, *Nucleic Acids Res.* 17:447-448 (1989)).

Briefly, the IiPADRE construct contains the full length Ii sequence with PADRE precisely replacing the CLIP region. The DNA construct encodes amino acids 1 through 87 of invariant chain, followed with the 13 amino acid PADRE sequence (SEQ ID NO:38) and the rest of the invariant chain DNA sequence
10 (amino acids 101-215). The construct was amplified in 2 overlapping halves that were joined to produce the final construct. The two primers used to amplify the 5' half were murli-F and the oligonucleotide
CAGGGTCCAGGCAGCCACGAACTTGGCCACAGGTTTGGCAGA (SEQ ID NO:42), which is designated IiPADRE-R. The IiPADRE-R primer includes nucleotides
15 303-262 of IiPADRE. The 3' half was amplified with the primer
GGCTGCCTGGACCCTGAAGGCTGCCGCTATGTCCATGGATAAC (SEQ ID NO:43), which is designated IiPADRE-F and includes nucleotides 288-330 of IiPADRE; and murli-R. The PCR conditions were the same as described above, and the two halves were isolated by agarose gel electrophoresis as described above.

20 Ten microliters of each PCR product was combined in a 100 µl PCR reaction with an annealing temperature of 50°C for five cycles to generate a full length template. Primers murli-F and murli-R were added and 25 more cycles carried out. The full length IiPADRE product was isolated, cloned, and sequenced as described above. This construct contains the murine Ii gene with a pan DR epitope sequence substituted for
25 the CLIP sequence of Ii (Figure 1).

A DNA construct, designated I80T, containing the cytoplasmic domain, the transmembrane domain and part of the luminal domain of Ii fused to a string of multiple MHC class II epitopes was constructed (Figure 2). Briefly, the string of multiple MHC class II epitopes was constructed with three overlapping oligonucleotides (oligos). Each
30 oligo overlapped its neighbor by 15 nucleotides and the final MHC class II epitope string was assembled by extending the overlapping oligonucleotides in three sets of reactions using PCR. The three oligonucleotides were: oligo 1, nucleotides 241-310,
CTTCGCATGAAGCTTATCAGCCAGGCTGTGCACGCCGCTCACGCCGAAATCAA
CGAAGCTGGAAGAACCC (SEQ ID NO:44);

oligo 2, nucleotides 364-295,

TTCTGGTCAGCAGAAAGAACAGGATAGGAGCGTTTGGAGGGCGATAAGCTGG
AGGGGTTCTTCCAGCTTC (SEQ ID NO:45); and

oligo 3, nucleotides 350-42,

5 TTCTGCTGACCAGAATCCTGACAATCCCCCAGTCCCTGGACGCCAAGTTCGTG
GCTGCCTGGACCCTGAAG (SEQ ID NO:46).

For the first PCR reaction, 5 µg of oligos 1 and 2 were combined in a 100 µl reaction containing *Pfu* polymerase. A Perkin/Elmer 9600 PCR machine was used and the annealing temperature used was 45° C. The PCR product was gel-purified, and a
10 second reaction containing the PCR product of oligos 1 and 2 with oligo 3 was annealed and extended for 10 cycles before gel purification of the full length product to be used as a "mega-primer."

The I80T construct was made by amplifying bli#3 with murli-F and the mega-primer. The cycling conditions were: 1 cycle of 95° C for 5 minutes, followed by 5
15 cycles of 95° C for 15 seconds, 37° C for 30 seconds, and 72° C for 1 minute. Primer Help-epR was added and an additional 25 cycles were carried out with the annealing temperature raised to 47° C. The Help-epR primer
GGTACCTCAAGCGGCAGCCTTCAGGGTCCAGGCA (SEQ ID NO:47) corresponds to nucleotides 438-405. The full length I80T product was isolated, cloned, and sequenced
20 as above.

The I80T construct (Figure 2) encodes amino acid residues 1 through 80 of Ii, containing the cytoplasmic domain, the transmembrane domain and part of the luminal domain, fused to a string of multiple MHC class II epitopes corresponding to: amino acid residues 323-339 of ovalbumin
25 (IleSerGlnAlaValHisAlaAlaHisAlaGluIleAsnGluAlaGlyArg; SEQ ID NO:48); amino acid residues 128 to 141 of HBV core antigen (amino acids ThrProProAlaTyrArgProProAsnAlaProIleLeu; SEQ ID NO:49); amino acid residues 182 to 196 of HBV env (amino acids PhePheLeuLeuThrArgIleLeuThrIleProGlnSerLeuAsp; SEQ ID NO:50); and the pan DR sequence designated SEQ ID NO:38.

30 A DNA construct containing the cytoplasmic domain, transmembrane domain and a portion of the luminal domain of Ii fused to the MHC class II epitope string shown in Figure 2 and amino acid residues 101 to 215 of Ii encoding the trimerization region of Ii was generated (Figure 3). This construct, designated IiThfull, encodes the first 80 amino acids of invariant chain followed by the MHC class II epitope string

(replacing CLIP) and the rest of the invariant chain (amino acids 101-215). Briefly, the construct was generated as two overlapping halves that were annealed and extended by PCR to yield the final product.

The 5' end of IiThfull was made by amplifying I80T with murIi-F (SEQ ID NO:40) and Th-Pad-R. The Th-Pad-R primer AGCGGCAGCCTTCAGGGTC (SEQ ID NO:51) corresponds to nucleotides 429-411. The 3' half was made by amplifying bIi#3 with IiPADRE-F and murIi-R (SEQ ID NO:41). The IiPADRE-F primer GGCTGCCTGGACCCTGAAGGCTGCCGCTATGTCCATGGATAAC (SEQ ID NO:52) corresponds to nucleotides 402-444. Each PCR product was gel purified and mixed, then denatured, annealed, and extended by five cycles of PCR. Primers murIi-F (SEQ ID NO:40) and murIi-R (SEQ ID NO:41) were added and another 25 cycles performed. The full length product was gel purified, cloned, and sequenced.

All of the remaining constructs described below were made essentially according to the scheme shown in Figure 18. Briefly, primer pairs 1F plus 1R, designated below for each specific construct, were used to amplify the specific signal sequence and contained an overlapping 15 base pair tail identical to the 5' end of the MHC class II epitope string. Primer pair Th-ova-F, ATCAGCCAGGCTGTGCACGC (SEQ ID NO:53), plus Th-Pad-R (SEQ ID NO:51) were used to amplify the MHC class II epitope string. A 15 base pair overlap and the specific transmembrane and cytoplasmic tail containing the targeting signals were amplified with primer pairs 2F plus 2R.

All three pieces of each cDNA were amplified using the following conditions: 1 cycle of 95°C for 5 minutes, followed by 30 cycles of 95°C for 15 seconds, 52°C for 30 seconds, and 72°C for 1 minute. Each of the three fragments was agarose-gel purified, and the signal sequence and MHC class II string fragments were combined and joined by five cycles in a second PCR. After five cycles, primers 1F and Th-Pad-R were added for 25 additional cycles and the PCR product was gel purified. This signal sequence plus MHC class II epitope string fragment was combined with the transmembrane plus cytoplasmic tail fragment for the final PCR. After five cycles, primers 1F plus 2R were added for 25 additional cycles and the product was gel purified, cloned and sequenced.

A DNA construct containing the murine immunoglobulin kappa signal sequence fused to the T helper epitope string shown in Figure 2 and the transmembrane and cytoplasmic domains of LAMP-1 was generated (Figure 4) (Granger *et al.*, *J. Biol. Chem.* 265:12036-12043 (1990)), (mouse LAMP-1)

GenBank accession No. M32015). This construct, designated kappaLAMP-Th, contains the consensus mouse immunoglobulin kappa signal sequence and was amplified from a plasmid containing full length immunoglobulin kappa as depicted in Figure 18. The primer 1F used was the oligonucleotide designated KappaSig-F,

5 GCTAGCGCCGCCACCATGGGAATGCAG (SEQ ID NO:54).

The primer 1R used was the oligonucleotide designated Kappa-Th-R, CACAGCCTGGCTGATTCCTCTGGACCC (SEQ ID NO:55).

The primer 2F used was the oligonucleotide designated PAD/LAMP-F, CTGAAGGCTGCCGCTAACAACATGTTGATCCCC (SEQ ID NO:56). The primer 2R used was the oligonucleotide designated LAMP-CYTOR, GGTACCCTAGATGGTCTGATAGCC (SEQ ID NO:57).

A DNA construct containing the signal sequence of H2-M fused to the MHC class II epitope string shown in Figure 2 and the transmembrane and cytoplasmic domains of H2-M was generated (Figure 5). The mouse H2-M gene has been described previously, Peleraux *et al.*, *Immunogenetics* 43:204-214 (1996)).

This construct was designated H2M-Th and was constructed as depicted in Figure 18. The primer 1F used was the oligonucleotide designated H2-Mb-1F, GCC GCT AGC GCC GCC ACC ATG GCT GCA CTC TGG (SEQ ID NO:58). The primer 1R used was the oligonucleotide designated H2-Mb-1R, CAC AGC CTG GCT GAT CCC CAT ACA GTG CAG (SEQ ID NO:59). The primer 2F used was the oligonucleotide designated H2-Mb-2F, CTG AAG GCT GCC GCT AAG GTC TCT GTG TCT (SEQ ID NO:60). The primer 2R used was the oligonucleotide designated H2-Mb-2R, GCG GGT ACC CTA ATG CCG TCC TTC (SEQ ID NO:61).

A DNA construct containing the signal sequence of H2-DO fused to the MHC class II epitope string shown in Figure 2 and the transmembrane and cytoplasmic domains of H2-DO was generated (Figure 6). The mouse H2-DO gene has been described previously (Larhammar *et al.*, *J. Biol. Chem.* 260:14111-14119 (1985)),

(GenBank accession No. M19423). This construct, designated H2O-Th, was constructed as depicted in Figure 18. The primer 1F used was the oligonucleotide designated H2-Ob-1F, GCG GCT AGC GCC GCC ACC ATG GGC GCT GGG AGG (SEQ ID NO:62). The primer 1R used was the oligonucleotide designated H2-Ob-1R, TGC ACA GCC TGG CTG ATG GAA TCC AGC CTC (SEQ ID NO:63). The primer 2F used was the oligonucleotide designated H2-Ob-2F, CTG AAG GCT GCC GCT ATA CTG AGT GGA GCT (SEQ ID NO:64). The primer 2R used was

the oligonucleotide designated H2-Ob-2R, GCC GGT ACC TCA TGT GAC ATG TCC CG (SEQ ID NO:65).

A DNA construct containing a pan DR epitope sequence (SEQ ID NO:38) fused to the amino-terminus of influenza matrix protein is generated (Figure 7). This construct, designated PADRE-Influenza matrix, contains the universal MHC class II epitope PADRE attached to the amino terminus of the influenza matrix coding sequence. The construct is made using a long primer on the 5' end primer. The 5' primer is the oligonucleotide
 GCTAGCGCCGCCACCATGGCCAAGTTCGTGGCTGCCTGGACCCTGAAGGCTGC
 10 CGCTATGAGTCTTCTAACCGAGGTCGA (SEQ ID NO:66). The 3' primer is the oligonucleotide TCACTTGAATCGCTGCATCTGCACCCCCAT (SEQ ID NO:67). Influenza virus from the America Type Tissue Collection (ATCC) is used as a source for the matrix coding region (Perdue *et al. Science* 279:393-396 (1998)),
 (GenBank accession No. AF036358).

15 A DNA construct containing a pan DR epitope sequence (SEQ ID NO:38) fused to the amino-terminus of HBV-S antigen was generated (Figure 8). This construct is designated PADRE-HBV-s and was generated by annealing two overlapping oligonucleotides to add PADRE onto the amino terminus of hepatitis B surface antigen (Michel *et al., Proc. Natl. Acad. Sci. USA* 81:7708-7712 (1984); Michel *et al., Proc. Natl. Acad. Sci. USA* 92:5307-5311 (1995)), each of which is incorporated herein by reference. One oligonucleotide was
 GCTAGCGCCGCCACCATGGCCAAGTTCGTGGCTGCCTGGACCCTGAAGGCTGC
 CGCTC (SEQ ID NO:68). The second oligonucleotide was
 CTCGAGAGCGGCAGCCTTCAGGGTCCAGGCAGCCACGAACTTGGCCATGGTG
 25 GCGGCG (SEQ ID NO:69). When annealed, the oligos have NheI and XhoI cohesive ends. The oligos were heated to 100°C and slowly cooled to room temperature to anneal. A three part ligation joined PADRE with an XhoI-KpnI fragment containing HBV-s antigen into the NheI plus KpnI sites of the expression vector.

A DNA construct containing the signal sequence of Ig- α fused to the MHC class II epitope string shown in Figure 2 and the transmembrane and cytoplasmic domains of Ig- α was generated (Figure 9). The mouse Ig- α gene has been described previously (Kashiwamura *et al., J. Immunol.* 145:337-343 (1990)),

(GenBank accession No. M31773). This construct, designated Ig-alphaTh, was constructed as depicted in Figure 18. The primer 1F used was the oligonucleotide

designated Ig alpha-1F, GCG GCT AGC GCC GCC ACC ATG CCA GGG GGT CTA (SEQ ID NO:70). The primer 1R used was the oligonucleotide designated Igalpha-1R, GCA CAG CCT GGC TGA TGG CCT GGC ATC CGG (SEQ ID NO:71). The primer 2F used was the oligonucleotide designated Igalpha-2F, CTG AAG GCT GCC GCT GGG
 5 ATC ATC TTG CTG (SEQ ID NO:72). The primer 2R used was the oligonucleotide designated Igalpha-2R, GCG GGT ACC TCA TGG CTT TTC CAG CTG (SEQ ID NO:73).

A DNA construct containing the signal sequence of Ig- β fused to the MHC class II string shown in Figure 2 and the transmembrane and cytoplasmic domains of Ig β was generated (Figure 10). The Ig- β sequence is the B29 gene of mouse and has been described previously (Hermanson *et al.*, *Proc. Natl. Acad. Sci. USA* 85:6890-6894 (1988)) (GenBank accession No. J03857).

This construct, designated Ig-betaTh, was constructed as depicted in Figure 18. The primer 1F used was the oligonucleotide designated B29-1F (33mer) GCG GCT AGC
 15 GCC GCC ACC ATG GCC ACA CTG GTG (SEQ ID NO:74). The primer 1R used was the oligonucleotide designated B29-1R (30mer) CAC AGC CTG GCT GAT CGG CTC ACC TGA GAA (SEQ ID NO:75). The primer 2F used was the oligonucleotide designated B292F (30mer) CTG AAG GCT GCC GCT ATT ATC TTG ATC CAG (SEQ ID NO: 76). The primer 2R used was the oligonucleotide designated B29-2R (27mer),
 20 GCC GGT ACC TCA TTC CTG GCC TGG ATG (SEQ ID NO:77).

A DNA construct containing the signal sequence of the kappa immunoglobulin signal sequence fused to the MHC class II epitope string shown in Figure 2 was constructed (Figure 11). This construct is designated SigTh and was generated by using the kappaLAMP-Th construct (shown in Figure 4) and amplifying
 25 with the primer pair KappaSig-F (SEQ ID NO:54) plus Help-epR (SEQ ID NO:47) to create SigTh. SigTh contains the kappa immunoglobulin signal sequence fused to the T helper epitope string and terminated with a translational stop codon.

Constructs encoding human sequences corresponding to the above described constructs having mouse sequences are prepared by substituting human
 30 sequences for the mouse sequences. Briefly, for the IiPADRE construct, corresponding to Figure 1, amino acid residues 1-80 from the human Ii gene HLA-DR sequence (Figure 12) (GenBank accession No. X00497 M14765) is substituted for the mouse Ii sequences, which is fused to PADRE, followed by human invariant chain HLA-DR amino acid residues 114-223. For the I80T construct, corresponding to Figure 2, amino acid residues

1-80 from the human sequence of Ii is followed by a MHC class II epitope string. For the IiThfull construct, corresponding to Figure 3, amino acid residues 1-80 from the human sequence of Ii, which is fused to a MHC class II epitope string, is followed by human invariant chain amino acid residues 114-223.

5 For the LAMP-Th construct, similar to Figure 4, the signal sequence encoded by amino acid residues 1-19 (nucleotides 11-67) of human LAMP-1 (Figure 13) (GenBank accession No. J04182), which is fused to the MHC class II epitope string, is followed by the transmembrane (nucleotides 1163-1213) and cytoplasmic tail (nucleotides 1214-1258) region encoded by amino acid residues 380-416 of human
10 LAMP-1.

For the HLA-DM-Th construct, corresponding to Figure 5, the signal sequence encoded by amino acid residues 1-17 (nucleotides 1-51) of human HLA-DMB (Figure 14) (GenBank accession No. U15085), which is fused to the MHC class II epitope string, is followed by the transmembrane (nucleotides 646-720) and cytoplasmic tail
15 (nucleotides 721-792) region encoded by amino acid residues 216-263 of human HLA-DMB.

For the HLA-DO-Th construct, corresponding to Figure 6, the signal sequence encoded by amino acid residues 1-21 (nucleotides 1-63) of human HLA-DO (Figure 15) (GenBank accession No. L29472 J02736 N00052), which is fused to the
20 MHC class II epitope string, is followed by the transmembrane (nucleotides 685-735) and cytoplasmic tail (nucleotides 736-819) region encoded by amino acid residues 223-273 of human HLA-DO.

For the Ig-alphaTh construct, corresponding to Figure 9, the signal sequence encoded by amino acid residues 1-29 (nucleotides 1-87) of human Ig-alpha MB-1
25 (Figure 16) (GenBank accession No. U05259), which is fused to the MHC class II epitope string, is followed by the transmembrane (nucleotides 424-498) and cytoplasmic tail (nucleotides 499-678) region encoded by amino acid residues 142-226 of human Ig-alpha MB-1.

For the Ig-betaTh construct, corresponding to Figure 10, the signal
30 sequence encoded by amino acid residues 1-28 (nucleotides 17-100) of human Ig-beta B29 (Figure 17) (GenBank accession No. M80461), which is fused to the MHC class II epitope string, is followed by the transmembrane (nucleotides 500-547) and cytoplasmic tail (nucleotides 548-703) region encoded by amino acid residues 156-229 of human Ig-beta.

The SigTh construct shown in Figure 11 can be used in mouse and human. Alternatively, a signal sequence derived from an appropriate human gene containing a signal sequence can be substituted for the mouse kappa immunoglobulin sequence in the Sig Th construct.

5 The PADRE-Influenza matrix construct shown in Figure 7 and the PADRE-HBVs construct shown in Figure 8 can be used in mouse and human.

Some of the DNA constructs described above were cloned into the vector pEP2 (Figure 19; SEQ ID NO:35). The pEP2 vector was constructed to contain dual CMV promoters. The pEP2 vector used the backbone of pcDNA3.1(-)Myc-His A from
10 Invitrogen and pIRES1hyg from Clontech. Changes were made to both vectors before the CMV transcription unit from pIRES1hyg was moved into the modified pcDNA vector.

The pcDNA3.1(-)Myc-His A vector (<http://www.invitrogen.com>) was modified. Briefly, the PvuII fragment (nucleotides 1342-3508) was deleted. A BspHI fragment that contains the Ampicillin resistance gene (nucleotides 4404-5412) was cut
15 out. The Ampicillin resistance gene was replaced with the kanamycin resistance gene from pUC4K (GenBank Accession #X06404). pUC4K was amplified with the primer set: TCTGATGTTACATTGCACAAG (SEQ ID NO:78) (nucleotides 1621-1601) and GCGCACTCATGATGCTCTGCCAGTGTTACAACC (SEQ ID NO:79) (nucleotides 682-702 plus the addition of a BspHI restriction site on the 5' end). The PCR product
20 was digested with BspHI and ligated into the vector digested with BspHI. The region between the PmeI site at nucleotide 905 and the EcoRV site at nucleotide 947 was deleted. The vector was then digested with PmeI (cuts at nucleotide 1076) and ApaI (cuts at nucleotide 1004), Klenow filled in at the cohesive ends and ligated. The KpnI site at nucleotide 994 was deleted by digesting with KpnI and filling in the ends with Klenow
25 DNA polymerase, and ligating. The intron A sequence from CMV (GenBank accession M21295, nucleotides 635-1461) was added by amplifying CMV DNA with the primer set: GCGTCTAGAGTAAGTACCGCCTATAGACTC (SEQ ID NO:80) (nucleotides 635-655 plus an XbaI site on the 5' end) and CCGGCTAGCCTGCAGAAAAGACCCATGGAA (SEQ ID NO:81) (nucleotides 1461-1441 plus an NheI site on the 3' end). The PCR
30 product was digested with XbaI and NheI and ligated into the NheI site of the vector (nucleotide 895 of the original pcDNA vector) so that the NheI site was on the 3' end of the intron.

To modify the pIRES1hyg vector (GenBank Accession U89672, Clontech), the KpnI site (nucleotide 911) was deleted by cutting and filling in with

Klenow. The plasmid was cut with NotI (nucleotide 1254) and XbaI (nucleotide 3196) and a polylinker oligo was inserted into the site. The polylinker was formed by annealing the following two oligos:

GGCCGCAAGGAAAAAATCTAGAGTCGGCCATAGACTAATGCCGGTACCG (SEQ
5 ID NO:82) and

CTAGCGGTACCGGCATTAGTCTATGGCCCGACTCTAGATTTTTTCCTTGC (SEQ
ID NO:83). The resulting plasmid was cut with HincII and the fragment between HincII
sites 234 and 3538 was isolated and ligated into the modified pcDNA vector. This
fragment contains a CMV promoter, intron, polylinker, and polyadenylation signal.

10 The pIRES1hyg piece and the pcDNA piece were combined to form pEP2.
The modified pcDNA3.1(-)Myc-His A vector was partially digested with PvuII to isolate
a linear fragment with the cut downstream of the pcDNA polyadenylation signal (the
other PvuII site is the CMV intron). The HincII fragment from the modified pIRES1hyg
vector was ligated into the PvuII cut vector. The polyadenylation signal from the pcDNA
15 derived transcription unit was deleted by digesting with EcoRI (pcDNA nucleotide 955)
and XhoI (pIRES1hyg nucleotide 3472) and replaced with a synthetic polyadenylation
sequence. The synthetic polyadenylation signal was described in Levitt *et al.*, *Genes and
Development* 3:1019-1025 (1989)).

Two oligos were annealed to produce a fragment that contained a
20 polylinker and polyadenylation signal with EcoRI and XhoI cohesive ends. The oligos
were:
AATTCGGATATCCAAGCTTGATGAATAAAAGATCAGAGCTCTAGTGATCTGTGT
GTTGGTTTTTTTGTGTGC (SEQ ID NO:84) and
TCGAGCACACAAAAACCAACACACAGATCACTAGAGCTCTGATCTTTTATT
25 CATCAAGCTTGGATATCCG (SEQ ID NO:85).

The resulting vector is named pEP2 and contains two separate
transcription units. Both transcription units use the same CMV promoter but each
contains different intron, polylinker, and polyadenylation sequences.

30 The pEP2 vector contains two transcription units. The first transcription
unit contains the CMV promoter initially from pcDNA (nucleotides 210-862 in Figure
19), CMV intron A sequence (nucleotides 900-1728 in Figure 19), polylinker cloning site
(nucleotides 1740-1760 in Figure 19) and synthetic polyadenylation signal (nucleotides
1764-1769 in Figure 19). The second transcription unit, which was initially derived from
pIRES1hyg, contains the CMV promoter (nucleotides 3165-2493 in Figure 19), intron

sequence (nucleotides 2464-2173 in Figure 19), polylinker clone site (nucleotides 2126-2095 in Figure 19) and bovine growth hormone polyadenylation signal (nucleotides 1979-1974 in Figure 19). The kanamycin resistance gene is encoded in nucleotides 4965-4061 (Figure 19).

5 The DNA constructs described above were digested with NheI and KpnI and cloned into the XbaI and KpnI sites of pEP2 (the second transcription unit).

Additional vectors were also constructed. To test for the effect of co-expression of MHC class I epitopes with MHC class II epitopes, an insert was generated, designated AOS, that contains nine MHC class I epitopes. The AOS insert was initially
10 constructed in the vector pMIN.0 (Figure 20; SEQ ID NO:36). Briefly, the AOS insert contains nine MHC class I epitopes, six restricted by HLA-A2 and three restricted by HLA-A11, and the universal MHC class II epitope PADRE. The vector pMIN.0 contains epitopes from HBV, HIV and a mouse ovalbumin epitope. The MHC class I epitopes appear in pMIN.0 in the following order:

15 consensus mouse Ig Kappa signal sequence (pMIN.0 amino acid residues 1-20, nucleotides 16-81) MQVQIQSLFLLLLWVPGSRG (SEQ ID NO:86) encoded by nucleotides ATG CAG GTG CAG ATC CAG AGC CTG TTT CTG CTC CTC CTG TGG GTG CCC GGG TCC AGA GGA (SEQ ID NO:87);

 HBV pol 149-159 (A11 restricted)
20 (pMIN.0 amino acid residues 21-31, nucleotides 82-114)
HTLWKAGILYK (SEQ ID NO:88) encoded by nucleotides CAC ACC CTG TGG AAG GCC GGA ATC CTG TAT AAG (SEQ ID NO:89);

 PADRE-universal MHC class II epitope (pMIN.0 amino acid residues 32-45, nucleotides 115-153) AKFVAAWTLKAAA (SEQ ID NO:38) encoded by nucleotides
25 GCC AAG TTC GTG GCT GCC TGG ACC CTG AAG GCT GCC GCT (SEQ ID NO:90);

 HBV core 18-27 (A2 restricted) (pMIN.0 amino acid residues 46-55, nucleotides 154-183) FLPSDFFPSV (SEQ ID NO:91) encoded by nucleotides TTC CTG CCT AGC GAT TTC TTT CCT AGC GTG (SEQ ID NO:92);

30 HIV env 120-128 (A2 restricted) (pMIN.0 amino acid residues 56-64, nucleotides 184-210) KLTPLCVTL (SEQ ID NO:93) encoded by nucleotides AAG CTG ACC CCA CTG TGC GTG ACC CTG (SEQ ID NO:94);

HBV pol 551-559 (A2 restricted) (pMIN.0 amino acid residues 65-73, nucleotides 211-237) YMDDVVLGA (SEQ ID NO:95) encoded by nucleotides TAT ATG GAT GAC GTG GTG CTG GGA GCC (SEQ ID NO:96);

5 mouse ovalbumin 257-264 (K^b restricted) (pMIN.0 amino acid residues 74-81, nucleotides 238-261) SHNFEKL (SEQ ID NO:97) encoded by nucleotides AGC ATC ATC AAC TTC GAG AAG CTG (SEQ ID NO:98);

HBV pol 455-463 (A2 restricted) (pMIN.0 amino acid residues 82-90, nucleotides 262-288) GLSRYVARL (SEQ ID NO:99) encoded by nucleotides GGA CTG TCC AGA TAC GTG GCT AGG CTG (SEQ ID NO:100);

10 HIV pol 476-84 (A2 restricted) (pMIN.0 amino acid residues 91-99, nucleotides 289-315) ILKEPVHGV (SEQ ID NO:101) encoded by nucleotides ATC CTG AAG GAG CCT GTG CAC GGC GTG (SEQ ID NO:102);

HBV core 141-151 (A11 restricted)

(pMIN.0 amino acid residues 100-110, nucleotides 316-348)
15 STLPETTVVRR (SEQ ID NO:103) encoded by nucleotides TCC ACC CTG CCA GAG ACC ACC GTG GTG AGG AGA (SEQ ID NO:104);

HIV env 49-58 (A11 restricted) (pMIN.0 amino acid residues 111-120, nucleotides 349-378) TVYYGVPVWK (SEQ ID NO:105) encoded by nucleotides ACC GTG TAC TAT GGA GTG CCT GTG TGG AAG (SEQ ID NO:106); and

20 HBV env 335-343 (A2 restricted) (pMIN.0 amino acid residues 121-129, nucleotides 378-405) WLSLLVPFV (SEQ ID NO:107) encoded by nucleotides TGG CTG AGC CTG CTG GTG CCC TTT GTG (SEQ ID NO:108).

The pMIN.0 vector contains a KpnI restriction site (pMIN.0 nucleotides 406-411) and a NheI restriction site (pMIN.0 nucleotides 1-6). The pMIN.0 vector
25 contains a consensus Kozak sequence (nucleotides 7-18) (GCCGCCACCATG; SEQ ID NO:109) and murine Kappa Ig-light chain signal sequence followed by a string of 10 MHC class I epitopes and one universal MHC class II epitope. The pMIN.0 sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector. The pMIN.0 vector was constructed with eight
30 oligonucleotides:

MinI oligo

GAGGAGCAGAAACAGGCTCTGGATCTGCACCTGCATTCCCATGGTGGCGGCGC
TAGCAAGCTTCTTGCGC (SEQ ID NO:110);

Min2 oligo

CCTGTTTCTGCTCCTCCTGTGGGTGCCCCGGGTCCAGAGGACACACCCTGTGGA
AGGCCGGAATCCTGTATA (SEQ ID NO:111);

Min3 oligo

5 TCGCTAGGCAGGAAAGCGGCAGCCTTCAGGGTCCAGGCAGCCACGAACTTGG
CCTTATACAGGATTCCGG (SEQ ID NO:112);

Min4 oligo

CTTTCCTGCCTAGCGATTTCTTTCCTAGCGTGAAGCTGACCCCACTGTGCGTGA
CCCTGTATATGGATGAC (SEQ ID NO:113);

10 Min5 oligo

CGTACCTGGACAGTCCCAGCTTCTCGAAGTTGATGATGCTGGCT
CCCAGCACACGTCATCCATATACAG (SEQ ID NO:114);

Min6 oligo

GGACTGTCCAGATACGTGGCTAGGCTGATCCTGAAGGAGCCTGTGCACGGCGT
15 GTCCACCCTGCCAGAGAC (SEQ ID NO:115);

Min7 oligo

GCTCAGCCACTTCCACACAGGCACTCCATAGTACACGGTCCTCCTCACCACGG
TGGTCTCTGGCAGGGTG (SEQ ID NO:116);

Min8 oligo

20 GTGGAAGTGGCTGAGCCTGCTGGTGCCCTTTGTGGGTACCTGATCTAGAGC
(SEQ ID NO:117).

Additional primers were flanking primer 5', GCG CAA GAA GCT TGC
TAG CG (SEQ ID NO:118) and flanking primer 3', GCT CTA GAT CAG GTA CCC
CAC (SEQ ID NO:119).

25 The original pMIN.0 minigene construction was carried out using eight
overlapping oligos averaging approximately 70 nucleotides in length, which were
synthesized and HPLC purified by Operon Technologies Inc. Each oligo overlapped its
neighbor by 15 nucleotides, and the final multi-epitope minigene was assembled by
extending the overlapping oligos in three sets of reactions using PCR (Ho *et al.*, *Gene*
30 77:51-59 (1989).

For the first PCR reaction, 5 µg of each of two oligos were annealed and
extended: 1+2, 3+4, 5+6, and 7+8 were combined in 100 µl reactions containing 0.25 mM
each dNTP and 2.5 units of Pfu polymerase in Pfu polymerase buffer containing 10 mM
KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% TRITON

X-100 and 100 mg/ml BSA. A Perkin/Elmer 9600 PCR machine was used and the annealing temperature used was 5°C below the lowest calculated T_m of each primer pair. The full length dimer products were gel-purified, and two reactions containing the product of 1-2 and 3-4, and the product of 5-6 and 7-8 were mixed, annealed and
 5 extended for 10 cycles. Half of the two reactions were then mixed, and 5 cycles of annealing and extension carried out before flanking primers were added to amplify the full length product for 25 additional cycles. The full length product was gel purified and cloned into pCR-blunt (Invitrogen) and individual clones were screened by sequencing. The Min insert was isolated as an NheI-KpnI fragment and cloned into the same sites of
 10 pcDNA3.1(-)/Myc-His A (Invitrogen) for expression. The Min protein contains the Myc and His antibody epitope tags at its carboxyl-terminal end.

For all the PCR reactions described, a total of 30 cycles were performed using Pfu polymerase and the following conditions: 95°C for 15 seconds, annealing temperature for 30 seconds, 72°C for one minute. The annealing temperature used was
 15 5°C below the lowest calculated T_m of each primer pair.

Three changes to pMIN.0 were made to produce pMIN.1 (Figure 21; SEQ ID NO:37, also referred to as pMIN-AOS). The mouse ova epitope was removed, the position 9 alanine anchor residue (#547) of HBV pol 551-560 was converted to a valine which increased the *in vitro* binding affinity 40-fold, and a translational stop codon was
 20 introduced at the end of the multi-epitope coding sequence. The changes were made by amplifying two overlapping fragments and combining them to yield the full length product.

The first reaction used the 5' pcDNA vector primer T7 and the primer Min-ovaR (nucleotides 247-218) TGGACAGTCCCAGTCCCAGCACCACGTCAT (SEQ ID
 25 NO:120). The 3' half was amplified with the primers: Min-ovaF (nucleotides 228-257) GCTGGGAGTGGGACTGTCCAGGTACGTGGC (SEQ ID NO:121) and Min-StopR (nucleotides 390-361) GGTACCTCACACAAAGGGCACCAGCAGGC (SEQ ID NO:122)

The two fragments were gel purified, mixed, denatured, annealed, and
 30 filled in with five cycles of PCR. The full length fragment was amplified with the flanking primers T7 and Min-Stop for 25 more cycles. The product was gel purified, digested with NheI and KpnI and cloned into pcDNA3.1 for sequencing and expression. The insert from pMin.1 was isolated as an NheI-KpnI fragment and cloned into pEP2 to make pEP2-AOS.

EXAMPLE II: Assay for T Helper Cell Activation

This example shows methods for assaying T helper cell activity. One method for assaying T helper cell activity uses spleen cells of an immunized organism.

5 Briefly, a spleen cell pellet is suspended with 2-3 ml of red blood cell lysis buffer containing 8.3 g/liter ammonium chloride in 0.001 M Tris-HCl, pH 7.5. The cells are incubated in lysis buffer for 3-5 min at room temperature with occasional vortexing. An excess volume of 50 ml of R10 medium is added to the cells, and the cells are pelleted. The cells are resuspended and pelleted one or two more times in R2 medium or R10
10 medium.

The cell pellet is suspended in R10 medium and counted. If the cell suspension is aggregated, the aggregates are removed by filtration or by allowing the aggregates to settle by gravity. The cell concentration is brought to 10^7 /ml, and 100 μ l of spleen cells are added to 96 well flat bottom plates.

15 Dilutions of the appropriate peptide, such as pan DR epitope (SEQ ID NO:145), are prepared in R10 medium at 100, 10, 1, 0.1 and 0.01 μ g/ml, and 100 μ l of peptide are added to duplicate or triplicate wells of spleen cells. The final peptide concentration is 50, 5, 0.5, 0.05 and 0.005 μ g/ml. Control wells receive 100 μ l R10 medium.

20 The plates are incubated for 3 days at 37°C. After 3 days, 20 μ l of 50 μ Ci/ml ^{3} H-thymidine is added per well. Cells are incubated for 18-24 hours and then harvested onto glass fiber filters. The incorporation of ^{3} H-thymidine into DNA of proliferating cells is measured in a beta counter.

A second assay for T helper cell activity uses peripheral blood
25 mononuclear cells (PBMC) that are stimulated *in vitro* as described in Alexander *et al.*, *supra* and Sette (WO 95/07,707), as adapted from Manca *et al.*, *J. Immunol.* 146:1964-1971 (1991). Briefly, PBMC are collected from healthy donors and purified over Ficoll-Plaque (Pharmacia Biotech; Piscataway, NJ). PBMC are plated in a 24 well tissue culture plate at 4×10^6 cells/ml. Peptides are
30 added at a final concentration of 10 μ g/ml. Cultures are incubated at 37°C in 5% CO₂.

On day 4, recombinant interleukin-2 (IL-2) is added at a final concentration of 10 ng/ml. Cultures are fed every 3 days by aspirating 1 ml of medium and replacing with fresh medium containing IL-2. Two additional stimulations of the T cells with antigen are performed on approximately days 14 and 28. The T cells ($3 \times$

10⁵/well) are stimulated with peptide (10 µg/ml) using autologous PBMC cells (2 x 10⁶ irradiated cells/well) (irradiated with 7500 rads) as antigen-presenting cells in a total of three wells of a 24 well tissue culture plate. In addition, on day 14 and 28, T cell proliferative responses are determined under the following conditions: 2 x 10⁴ T
 5 cells/well; 1 x 10⁵ irradiated PBMC/well as antigen-presenting cells; peptide concentration varying between 0.01 and 10 µg/ml final concentration. The proliferation of the T cells is measured 3 days later by the addition of ³H-thymidine (1 µCi/well) 18 hr prior to harvesting the cells. Cells are harvested onto glass filters and ³H-thymidine incorporation is measured in a beta plate counter. These results demonstrate methods for
 10 assaying T helper cell activity by measuring ³H-thymidine incorporation.

EXAMPLE III: Assay for Cytotoxic T Lymphocyte Response

This example shows a method for assaying cytotoxic T lymphocyte (CTL) activity. A CTL response is measured essentially as described previously (Vitiello *et al.*,
 15 *Eur. J. Immunol.* 27:671-678 (1997), which is incorporated herein by reference). Briefly, after approximately 10-35 days following DNA immunization, splenocytes from an animal are isolated and co-cultured at 37°C with syngeneic, irradiated (3000 rad) peptide-coated LPS blasts (1 x 10⁶ to 1.5 x 10⁶ cells/ml) in 10 ml R10 in T25 flasks. LPS blasts are obtained by activating splenocytes (1 x 10⁶ to 1.5 x 10⁶ cells/ml) with 25 µg/ml
 20 lipopolysaccharides (LPS) (Sigma cat. no. L-2387; St. Louis, MO) and 7 µg/ml dextran sulfate (Pharmacia Biotech) in 30 ml R10 medium in T75 flasks for 3 days at 37°C. The lymphoblasts are then resuspended at a concentration of 2.5 x 10⁷ to 3.0 x 10⁷/ml, irradiated (3000 rad), and coated with the appropriate peptides (100µg/ml) for 1 h at 37°C. Cells are washed once, resuspended in R10 medium at the desired concentration
 25 and added to the responder cell preparation. Cultures are assayed for cytolytic activity on day 7 in a ⁵¹Cr-release assay.

For the ⁵¹Cr-release assay, target cells are labeled for 90 min at 37°C with 150 µl sodium ⁵¹chromate (⁵¹Cr) (New England Nuclear; Wilmington DE), washed three times and resuspended at the appropriate concentration in R10 medium. For the assay,
 30 10⁴ target cells are incubated in the presence of different concentrations of effector cells in a final volume of 200 µl in U-bottom 96 well plates in the presence or absence of 10 µg/ml peptide. Supernatants are removed after 6 h at 37°C, and the percent specific lysis is determined by the formula: percent specific lysis = 100 x (experimental release - spontaneous release) / (maximum release - spontaneous release). To facilitate comparison

of responses from different experiments, the percent release data is transformed to lytic units 30 per 10^6 cells (LU30/ 10^6), with 1 LU30 defined as the number of effector cells required to induce 30% lysis of 10^4 target cells in a 6 h assay. LU values represent the LU30/ 10^6 obtained in the presence of peptide minus LU30/ 10^6 in the absence of peptide.

5 These results demonstrate methods for assaying CTL activity by measuring ^{51}Cr release from cells.

EXAMPLE IV: T Cell Proliferation in Mice Immunized with Expression Vectors
Encoding MHC Class II Epitopes and MHC Class II Targeting Sequences

10 This example demonstrates that expression vectors encoding MHC class II epitopes and MHC class II targeting sequences are effective at activating T cells.

The constructs used in the T cell proliferation assay are described in Example I and were cloned into the vector pEP2, a CMV driven expression vector. The peptides used for T cell *in vitro* stimulation are: Ova 323-339, ISQAVHAAHAEINEAGR
 15 (SEQ ID NO:123); HBVcore128, TPPAYRPPNAPILF (SEQ ID NO:124); HBVenv182, FFLTRILTIPQSLD (SEQ ID NO:125); and PADRE, AKFVAAWTLKAAA (SEQ ID NO:38).

T cell proliferation was assayed essentially as described in Example II. Briefly, 12 to 16 week old B6D2 F1 mice (2 mice per construct) were injected with 100
 20 μg of the indicated expression vector (50 μg per leg) in the anterior tibialis muscle. After eleven days, spleens were collected from the mice and separated into a single cell suspension by Dounce homogenization. The splenocytes were counted and one million splenocytes were plated per well in a 96-well plate. Each sample was done in triplicate. Ten $\mu\text{g}/\text{ml}$ of the corresponding peptide encoded by the respective expression vectors was
 25 added to each well. One well contained splenocytes without peptide added for a negative control. Cells were cultured at 37°C , 5% CO_2 for three days.

After three days, one μCi of ^3H -thymidine was added to each well. After 18 hours at 37°C , the cells were harvested onto glass filters and ^3H incorporation was measured on an LKB β plate counter. The results of the T cell proliferation assay are
 30 shown in Table 9. Antigen specific T cell proliferation is presented as the stimulation index (SI); this is defined as the ratio of the average ^3H -thymidine incorporation in the presence of antigen divided by the ^3H -thymidine incorporation in the absence of antigen.

The immunogen "PADRE + IFA" is a positive control where the PADRE peptide in incomplete Freund's adjuvant was injected into the mice and compared to the

response seen by injecting the MHC class II epitope constructs containing a PADRE sequence. As shown in Table 9, most of the expression vectors tested were effective at activating T cell proliferation in response to the addition of PADRE peptide. The activity of several of the expression vectors was comparable to that seen with immunization with the PADRE peptide in incomplete Freund's adjuvant. The expression vectors containing both MHC class I and MHC class II epitopes, pEP2-AOS and pcDNA-AOS, were also effective at activating T cell proliferation in response to the addition of PADRE peptide.

These results show that expression vectors encoding MHC class II epitopes fused to a MHC class II targeting sequence is effective at activating T cell proliferation and are useful for stimulating an immune response.

EXAMPLE V: *In vivo* assay Using Transgenic Mice

A. Materials and methods

Peptides were synthesized according to standard F-moc solid phase synthesis methods which have been previously described (Ruppert *et al.*, *Cell* 74:929 (1993); Sette *et al.*, *Mol. Immunol.* 31:813 (1994)). Peptide purity was determined by analytical reverse-phase HPLC and purity was routinely >95%. Synthesis and purification of the Theradigm-HBV lipopeptide vaccine is described in (Vitiello *et al.*, *J. Clin. Invest.* 95:341 (1995)).

Mice

HLA-A2.1 transgenic mice used in this study were the F1 generation derived by crossing transgenic mice expressing a chimeric gene consisting of the $\alpha 1$, $\alpha 2$ domains of HLA-A2.1 and $\alpha 3$ domain of H-2K^b with SJL/J mice (Jackson Laboratory, Bar Harbor, ME). This strain will be referred to hereafter as HLA-A2.1/K^b-H-2^{bx5}. The parental HLA-A2.1/K^b transgenic strain was generated on a C57BL/6 background using the transgene and methods described in (Vitiello *et al.*, *J. Exp. Med.* 173:1007 (1991)). HLA-A11/K^b transgenic mice used in the current study were identical to those described in (Alexander *et al.*, *J. Immunol.* 159:4753 (1997)).

Cell lines, MHC purification, and peptide binding assay

Target cells for peptide-specific cytotoxicity assays were Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (Vitiello *et al.*, *J. Exp. Med.* 173:1007

(1991)) and .221 tumor cells transfected with HLA-A11/K^b (Alexander *et al.*, *J. Immunol.* 159:4753 (1997)).

To measure presentation of endogenously processed epitopes, Jurkat-A2.1/K^b cells were transfected with the pMin.1 or pMin.2-GFP minigenes then tested in a cytotoxicity assay against epitope-specific CTL lines. For transfection, Jurkat-A2.1/K^b cells were resuspended at 10⁷ cells/ml and 30 µg of DNA was added to 600 µl of cell suspension. After electroporating cells in a 0.4 cm cuvette at 0.25 kV, 960 µF, cells were incubated on ice for 10 min then cultured for 2 d in RPMI culture medium. Cells were then cultured in medium containing 200 U/ml hygromycin B (Calbiochem, San Diego CA) to select for stable transfectants. FACS was used to enrich the fraction of green fluorescent protein (GFP)-expressing cells from 15% to 60% (data not shown).

Methods for measuring the quantitative binding of peptides to purified HLA-A2.1 and -A11 molecules is described in Ruppert *et al.*, *Cell* 74:929 (1993); Sette *et al.*, *Mol. Immunol.* 31:813 (1994); Alexander *et al.*, *J. Immunol.* 159:4753 (1997).

All tumor cell lines and splenic CTLs from primed mice were grown in culture medium (CM) that consisted of RPMI 1640 medium with Hepes (Life Technologies, Grand Island, NY) supplemented with 10% FBS, 4 mM L-glutamine, 5 X 10⁻⁵ M 2-ME, 0.5 mM sodium pyruvate, 100 µg/ml streptomycin, and 100 U/ml penicillin.

20

Construction of minigene multi-epitope DNA plasmids

pMIN.0 and pMIN.1 (i.e., pMIN-AOS) were constructed as described above,

25

pMin.1-No PADRE and pMin.1-Anchor. pMin.1 was amplified using two overlapping fragments which was then combined to yield the full length product. The first reaction used the 5' pcDNA vector primer T7 and either primer ATCGCTAGGCAGGAAGTTATACAGGATTCC (SEQ ID NO:126) for pMin.1-No PADRE or TGGACAGTCCGGCTCCCAGCACACGT (SEQ ID NO:127) for pMin.1-Anchor. The 3' half was amplified with the primers TTCCTGCCTAGCGATTTC (SEQ ID NO:128) (No PADRE) or GCTGGGAGCCGGACTGTCCAGGTACGT (SEQ ID NO:129) (Anchor) and Min-StopR. The two fragments generated from amplifying the 5' and 3' ends were gel purified, mixed, denatured, annealed, and filled in with five cycles

30

of PCR. The full length fragment was further amplified with the flanking primers T7 and Min-StopR for 25 more cycles.

pMin.1-No.Sig. The Ig signal sequence was deleted from pMin.1 by PCR amplification with primer GCTAGCGCCGCCACCATGCACACCCTGTGGAAGGC CGGAATC (SEQ ID NO:130) and pcDNA rev (Invitrogen) primers. The product was cloned into pCR-blunt and sequenced.

pMin.1-Switch. Three overlapping fragments were amplified from pMin.1, combined, and extended. The 5' fragment was amplified with the vector primer T7 and primer GGGC.ACCAGCAGGCTCAGCCACACTCCCAGCACCACGTC (SEQ ID NO:131). The second overlapping fragment was amplified with primers AGCCTGCTGGTGCCCTTTGTGATCCTGAAGGAGCCTGTGC (SEQ ID NO:132) and AGCCACGTACCTGGACAGTCCCTTCCACACAGGCACTCCAT (SEQ ID NO:133). Primer TGTCCAGGTACGTGGCTAGGCTGTGAGGTACC (SEQ ID NO:134) and the vector primer pcDNA rev (Invitrogen) were used to amplify the third (3') fragment. Fragments 1, 2, and 3 were amplified and gel purified. Fragments 2 and 3 were mixed, annealed, amplified, and gel purified. Fragment 1 was combined with the product of 2 and 3, and extended, gel purified and cloned into pcDNA3.1 for expression.

20

pMin.2-GFP. The signal sequence was deleted from pMin.0 by PCR amplification with Min.0-No Sig-5' plus pcDNA rev (Invitrogen) primers GCTAGCGCCGCCACCATGCACACCCTGTGGAAGGCCGGAATC (SEQ ID NO:135). The product was cloned into pCR-blunt and sequenced. The insert containing the open reading frame of the signal sequence-deleted multi-epitope construct was cut out with *NheI* plus *HindIII* and ligated into the same sites of pEGFPN1 (Clontech). This construct fuses the coding region of the signal-deleted pMin.0 construct to the N-terminus of green fluorescent protein (GFP).

30

Immunization of mice

For DNA immunization, mice were pretreated by injecting 50 μ l of 10 μ M cardiotoxin (Sigma Chem. Co., #C9759) bilaterally into the tibialis anterior muscle. Four or five days later, 100 μ g of DNA diluted in PBS were injected in the same muscle.

Theradigm-HBV lipopeptide (10 mg/ml in DMSO) that was stored at -20°C, was thawed for 10 min at 45°C before being diluted 1:10 (v/v) with room temperature PBS. Immediately upon addition of PBS, the lipopeptide suspension was vortexed vigorously and 100 µl was injected s.c. at the tail base (100 µg/mouse).

5 Immunogenicity of individual CTL epitopes was tested by mixing each CTL epitope (50 µg/mouse) with the HBV core 128-140 peptide (TPPAYRPPNAPIL (SEQ ID NO:124), 140 µg/mouse) which served to induce I-A^b-restricted Th cells. The peptide cocktail was then emulsified in incomplete Freund's adjuvant (Sigma Chem. Co.) and 100 µl of peptide emulsion was injected s.c. at the tail base.

10

In vitro CTL cultures and cytotoxicity assays

Eleven to 14 days after immunization, animals were sacrificed and a single cell suspension of splenocytes prepared. Splenocytes from cDNA-primed animals were stimulated *in vitro* with each of the peptide epitopes represented in the minigene.

15 Splenocytes ($2.5\text{--}3.0 \times 10^7$ /flask) were cultured in upright 25 cm² flasks in the presence of 10 µg/ml peptide and 10^7 irradiated spleen cells that had been activated for 3 days with LPS (25 µg/ml) and dextran sulfate (7 µg/ml). Triplicate cultures were stimulated with each epitope. Five days later, cultures were fed with fresh CM. After 10 d of *in vitro* culture, $2\text{--}4 \times 10^6$ CTLs from each flask were restimulated with 10^7 LPS/dextran sulfate-

20 activated splenocytes treated with 100 µg/ml peptide for 60-75 min at 37°C, then irradiated 3500 rads. CTLs were restimulated in 6-well plates in 8 ml of cytokine-free CM. Eighteen hr later, cultures received cytokines contained in con A-activated splenocyte supernatant (10-15% final concentration, v/v) and were fed or expanded on the third day with CM containing 10-15% cytokine supernate. Five days after restimulation,

25 CTL activity of each culture was measured by incubating varying numbers of CTLs with 10^4 ⁵¹Cr-labelled target cells in the presence or absence of peptide. To decrease nonspecific cytotoxicity from NK cells, YAC-1 cells (ATCC) were also added at a YAC-1:⁵¹Cr-labeled target cell ratio of 20:1. CTL activity against the HBV Pol 551 epitope was measured by stimulating DNA-primed splenocytes *in vitro* with the native A-

30 containing peptide and testing for cytotoxic activity against the same peptide.

To more readily compare responses, the standard E:T ratio vs % cytotoxicity data curves were converted into LU per 10^6 effector cells with one LU defined as the lytic activity required to achieve 30% lysis of target cells at a 100:1 E:T

ratio. Specific CTL activity (Δ LU) was calculated by subtracting the LU value obtained in the absence of peptide from the LU value obtained with peptide. A given culture was scored positive for CTL induction if all of the following criteria were met: 1) Δ LU > 2; 2) LU(+ peptide) $\div$ LU(- peptide) > 3; and 3) a >10% difference in % cytotoxicity tested with and without peptide at the two highest E:T ratios (starting E:T ratios were routinely between 25-50:1).

CTL lines were generated from pMin.1-primed splenocytes through repeated weekly stimulations of CTLs with peptide-treated LPS/DxS-activated splenocytes using the 6-well culture conditions described above with the exception that CTLs were expanded in cytokine-containing CM as necessary during the seven day stimulation period.

Cytokine assay

To measure IFN- γ production in response to minigene-transfected target cells, 4×10^4 CTLs were cultured with an equivalent number of minigene-transfected Jurkat-A2.1/K^b cells in 96-well flat bottom plates. After overnight incubation at 37°C, culture supernatant from each well was collected and assayed for IFN- γ concentration using a sandwich ELISA. Immulon II microtiter wells (Dynatech, Boston, MA) were coated overnight at 4°C with 0.2 μ g of anti-mouse IFN- γ capture Ab, R4-6A2 (Pharmingen). After washing wells with PBS/0.1% Tween-20 and blocking with 1% BSA, Ab-coated wells were incubated with culture supernate samples for 2 hr at room temperature. A secondary anti-IFN- γ Ab, XMGI.2 (Pharmingen), was added to wells and allowed to incubate for 2 hr at room temperature. Wells were then developed by incubations with Avidin-DH and finally with biotinylated horseradish peroxidase H (Vectastain ABC kit, Vector Labs, Burlingame, CA) and TMB peroxidase substrate (Kirkegaard and Perry Labs, Gaithersburg, MD). The amount of cytokine present in each sample was calculated using a rIFN- γ standard (Pharmingen).

b. Results

Selection of epitopes and minigene construct design

In the first series of experiments, the issue was whether a balanced multispecific CTL response could be induced by simple minigene cDNA constructs that encode several dominant HLA class I-restricted epitopes. Accordingly, nine CTL

epitopes were chosen on the basis of their relevance in CTL immunity during HBV and HIV infection in humans, their sequence conservancy among viral subtypes, and their class I MHC binding affinity (Table 10). Of these nine epitopes, six are restricted by HLA-A2.1 and three showed HLA-A11-restriction. One epitope, HBV Pol 551, was studied in two alternative forms: either the wild type sequence or an analog (HBV Pol 551-V) engineered for higher binding affinity.

As referenced in Table 10, several independent laboratories have reported that these epitopes are part of the dominant CTL response during HBV or HIV infection. All of the epitopes considered showed greater than 75% conservancy in primary amino acid sequence among the different HBV subtypes and HIV clades. The MHC binding affinity of the peptides was also considered in selection of the epitopes. These experiment addressed the feasibility of immunizing with epitopes possessing a wide range of affinities and, as shown in Table 10, the six HBV and three HIV HLA-restricted epitopes covered a spectrum of MHC binding affinities spanning over two orders of magnitude, with IC₅₀% concentrations ranging from 3 nM to 200 nM.

The immunogenicity of the six A2.1- and three A11-restricted CTL epitopes in transgenic mice was verified by co-immunization with a helper T cell peptide in an IFA formulation. All of the epitopes induced significant CTL responses in the 5 to 73 ΔLU range (Table 10). As mentioned above, to improve the MHC binding and immunogenicity of HBV Pol 551, the C-terminal A residue of this epitope was substituted with V resulting in a dramatic 40-fold increase in binding affinity to HLA-A2.1 (Table 10). While the parental sequence was weakly or nonimmunogenic in HLA transgenic mice, the HBV Pol 551-V analog induced significant levels of CTL activity when administered in IFA (Table 10). On the basis of these results, the V analog of the HBV Pol 551 epitope was selected for the initial minigene construct. In all of the experiments reported herein, CTL responses were measured with target cells coated with the native HBV Pol 551 epitope, irrespective of whether the V analog or native epitope was utilized for immunization.

Finally, since previous studies indicated that induction of T cell help significantly improved the magnitude and duration of CTL responses (Vitiello *et al.*, *J. Clin. Invest.* 95:341 (1995); Livingston *et al.*, *J. Immunol.* 159:1383 (1997)), the universal Th cell epitope PADRE was also incorporated into the minigene. PADRE has been shown previously to have high MHC binding affinity to a wide range of mouse and

human MHC class II haplotypes (Alexander *et al.*, *Immunity* 1:751 (1994)). In particular, it has been previously shown that PADRE is highly immunogenic in H-2^b mice that are used in the current study (Alexander *et al.*, *Immunity* 1:751 (1994)).

pMin.1, the prototype cDNA minigene construct encoding nine CTL epitopes and PADRE, was synthesized and subcloned into the pcDNA3.1 vector. The position of each of the nine epitopes in the minigene was optimized to avoid junctional mouse H-2^b and HLA-A2.1 class I MHC epitopes. The mouse Ig κ signal sequence was also included at the 5' end of the construct to facilitate processing of the CTL epitopes in the endoplasmic reticulum (ER) as reported by others (Anderson *et al.*, *J. Exp. Med.* 174:489 (1991)). To avoid further conformational structure in the translated polypeptide gene product that may affect processing of the CTL epitopes, an ATG stop codon was introduced at the 3' end of the minigene construct upstream of the coding region for c-myc and poly-his epitopes in the pcDNA3.1 vector.

15 Immunogenicity of pMin.1 in transgenic mice

To assess the capacity of the pMin.1 minigene construct to induce CTLs *in vivo*, HLA-A2.1/K^b-H-2^{bxs} transgenic mice were immunized intramuscularly with 100 μ g of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a control group of animals was also immunized with Theradigm-HBV, a palmitoylated lipopeptide consisting of the HBV Core 18 CTL epitope linked to the tetanus toxin 830-843 Th cell epitope.

Splenocytes from immunized animals were stimulated twice with each of the peptide epitopes encoded in the minigene, then assayed for peptide-specific cytotoxic activity in a ⁵¹Cr release assay. A representative panel of CTL responses of pMin.1-primed splenocytes, shown in Figure 22, clearly indicates that significant levels of CTL induction were generated by minigene immunization. The majority of the cultures stimulated with the different epitopes exceeded 50% specific lysis of target cells at an E:T ratio of 1:1. The results of four independent experiments, compiled in Table 11, indicate that the pMin.1 construct is indeed highly immunogenic in HLA-A2.1/K^b-H-2^{bxs} transgenic mice, inducing a broad CTL response directed against each of its six A2.1-restricted epitopes.

To more conveniently compare levels of CTL induction among the different epitopes, the % cytotoxicity values for each splenocyte culture was converted to

5 Δ LU and the mean Δ LU of CTL activity in positive cultures for each epitope was determined (see Example V, materials and methods, for positive criteria). The data, expressed in this manner in Table 11, confirms the breadth of CTL induction elicited by pMin.1 immunization since extremely high CTL responses, ranging between 50 to 700 Δ LU, were observed against the six A2.1-restricted epitopes. More significantly, the responses of several hundred Δ LU observed for five of the six epitopes approached or exceeded that of the Theradigm-HBV lipopeptide, a vaccine formulation known for its high CTL-inducing potency (Vitiello *et al.*, *J. Clin. Invest.* 95:341 (1995); Livingston *et al.*, *J. Immunol.* 159:1383 (1997)). The HBV Env 335 epitope was the only epitope
 10 showing a lower mean Δ LU response compared to lipopeptide (Table 11, 44 vs 349 Δ LU).

Processing of minigene epitopes by transfected cells

The decreased CTL response observed against HBV Env 335 was
 15 somewhat unexpected since this epitope had good A2.1 binding affinity (IC₅₀%, 5 nM) and was also immunogenic when administered in IFA. The lower response may be due, at least in part, to the inefficient processing of this epitope from the minigene polypeptide by antigen presenting cells following *in vivo* cDNA immunization. To address this possibility, Jurkat-A2.1 K^b tumor cells were transfected with pMin.1 cDNA and the
 20 presentation of the HBV Env 335 epitope by transfected cells was compared to more immunogenic A2.1-restricted epitopes using specific CTL lines. Epitope presentation was also studied using tumor cells transfected with a control cDNA construct, pMin.2-GFP, that encoded a similar multi-epitope minigene fused with GFP which allows detection of minigene expression in transfected cells by FACS.

25 Epitope presentation of the transfected Jurkat cells was analyzed using specific CTL lines, with cytotoxicity or IFN- γ production serving as a read-out. It was found that the levels of CTL response correlated directly with the *in vivo* immunogenicity of the epitopes. Highly immunogenic epitopes *in vivo*, such as HBV Core 18, HIV Pol 476, and HBV Pol 455, were efficiently presented to CTL lines by pMin.1- or pMin.2-
 30 GFP-transfected cells as measured by IFN- γ production (Figure 23A, >100 pg/ml for each epitope) or cytotoxic activity (Figure 23C, >30% specific lysis). In contrast to these high levels of *in vitro* activity, the stimulation of the HBV Env 335-specific CTL line against both populations of transfected cells resulted in less than 12 pg/ml IFN- γ and 3% specific

lysis. Although the HBV Env 335-specific CTL line did not recognize the naturally processed epitope efficiently, this line did show an equivalent response to peptide-loaded target cells, as compared to CTL lines specific for the other epitopes (Figure 23B, D). Collectively, these results suggest that a processing and/or presentation defect associated with the HBV Env 335 epitope that may contribute to its diminished immunogenicity *in vivo*.

Effect of the helper T cell epitope PADRE on minigene immunogenicity

Having obtained a broad and balanced CTL response in transgenic mice immunized with a minigene cDNA encoding multiple HLA-A2.1-restricted epitopes, next possible variables were examined that could influence the immunogenicity of the prototype construct. This type of analysis could lead to rational and rapid optimization of future constructs. More specifically, a cDNA construct based on the pMin.1 prototype was synthesized in which the PADRE epitope was deleted to examine the contribution of T cell help in minigene immunogenicity (Figure 24A).

The results of the immunogenicity analysis indicated that deletion of the PADRE Th cell epitope resulted in significant decreases in the frequency of specific CTL precursors against four of the minigene epitopes (HBV Core 18, HIV Env 120, HBV Pol 455, and HBV Env 335) as indicated by the 17 to 50% CTL-positive cultures observed against these epitopes compared to the 90-100% frequency in animals immunized with the prototype pMin.1 construct (Figure 25). Moreover, for two of the epitopes, HBV Core 18 and HIV Env 120, the magnitude of response in positive cultures induced by pMin.1-No PADRE was 20- to 30-fold less than that of the pMin.1 construct (Figure 25A).

Effect of modulation of MHC binding affinity on epitope immunogenicity

Next a construct was synthesized in which the V anchor residue in HBV Pol 551 was replaced with alanine, the native residue, to address the effect of decreasing MHC binding on epitope immunogenicity (Figure 24B).

Unlike deletion of the Th cell epitope, decreasing the MHC binding capacity of the HBV Pol 551 epitope by 40-fold through modification of the anchor residue did not appear to affect epitope immunogenicity (Figure 25B). The CTL response against the HBV Pol 551 epitope, as well as to the other epitopes, measured either by LU or frequency of CTL-positive cultures, was very similar between the constructs

containing the native A or improved V residue at the MHC binding anchor site. This finding reinforces the notion that minimal epitope minigenes can efficiently deliver epitopes of vastly different MHC binding affinities. Furthermore, this finding is particularly relevant to enhancing epitope immunogenicity via different delivery methods, especially in light of the fact that the wild type HBV Pol 551 epitope was essentially nonimmunogenic when delivered in a less potent IFA emulsion.

Effect of the signal sequence on minigene construct immunogenicity

The signal sequence was deleted from the pMin.1 construct, thereby preventing processing of the minigene polypeptide in the ER (Figure 24C). When the immunogenicity of the pMin.1-No Sig construct was examined, an overall decrease in response was found against four CTL epitopes. Two of these epitopes, HIV Env 120 and HBV Env 335, showed a decrease in frequency of CTL-positive cultures compared to pMin.1 while the remaining epitopes, HBV Pol 455 and HIV Pol 476, showed a 16-fold (from 424 to 27 Δ LU) and 3-fold decrease (709 to 236 Δ LU) in magnitude of the mean CTL response, respectively (Figure 25C). These findings suggest that allowing ER-processing of some of the epitopes encoded in the pMin.1 prototype construct may improve immunogenicity, as compared with constructs that allow only cytoplasmic processing of the same panel of epitopes.

Effect of epitope rearrangement and creation of new junctional epitopes

In the final construct tested, the immunogenicity of the HBV Env 335 epitope was analyzed to determine whether it may be influenced by its position at the 3' terminus of the minigene construct (Figure 24D). Thus, the position of the Env epitope in the cDNA construct was switched with a more immunogenic epitope, HBV Pol 455, located in the center of the minigene. It should be noted that this modification also created two potentially new epitopes. As shown in Figure 25D, the transposition of the two epitopes appeared to affect the immunogenicity of not only the transposed epitopes but also more globally of other epitopes. Switching epitopes resulted in obliteration of CTL induction against HBV Env 335 (no positive cultures detected out of six). The CTL response induced by the terminal HBV Pol 455 epitope was also decreased but only slightly (424 vs 78 mean Δ LU). In addition to the switched epitopes, CTL induction against other epitopes in the pMin.1-Switch construct was also markedly reduced

compared to the prototype construct. For example, a CTL response was not observed against the HIV Env 120 epitope and it was significantly diminished against the HBV Core 18 (4 of 6 positive cultures, decrease in mean Δ LU from 306 to 52) and HBV Pol 476 (decrease in mean Δ LU from 709 to 20) epitopes (Figure 25D).

5 As previously mentioned, it should be noted that switching the two epitopes had created new junctional epitopes. Indeed, in the pMin.1-Switch construct, two new potential CTL epitopes were created from sequences of HBV Env 335-HIV Pol 476 (LLVPFVIL (SEQ ID NO:135), H-2K^b-restricted) and HBV Env 335-HBV Pol 551 (VLGVWLSLLV (SEQ ID NO:136), HLA-A2.1-restricted) epitopes. Although these
10 junctional epitopes have not been examined to determine whether or not they are indeed immunogenic, this may account for the low immunogenicity of the HBV Env 335 and HIV Pol 476 epitopes. These findings suggest that avoiding junctional epitopes may be important in designing multi-epitope minigenes as is the ability to confirm their immunogenicity *in vivo* in a biological assay system such as HLA transgenic mice.

15

Induction of CTLs against A11 epitopes encoded in pMin.1

To further examine the flexibility of the minigene vaccine approach for inducing a broad CTL response against not only multiple epitopes but also against epitopes restricted by different HLA alleles, HLA-A11/K^b transgenic mice were
20 immunized to determine whether the three A11 epitopes in the pMin.1 construct were immunogenic for CTLs, as was the case for the A2.1-restricted epitopes in the same construct. As summarized in Table 12, significant CTL induction was observed in a majority of cultures against all three of the HLA-A11-restricted epitopes and the level of CTL immunity induced for the three epitopes, in the range of 40 to 260 Δ LU, exceeded
25 that of peptides delivered in IFA (Table 10). Thus, nine CTL epitopes of varying HLA restrictions incorporated into a prototype minigene construct all demonstrated significant CTL induction *in vivo*, confirming that minigene DNA plasmids can serve as means of delivering multiple epitopes, of varying HLA restrictions and MHC binding affinities, to the immune system in an immunogenic fashion and that appropriate transgenic mouse
30 strains can be used to measure DNA construct immunogenicity *in vivo*.

CTLs were also induced against three A11 epitopes in A11/K^b transgenic mice. These responses suggest that minigene delivery of multiple CTL epitopes that confers broad population coverage may be possible in humans and that transgenic animals

of appropriate haplotypes may be a useful tools in optimizing the *in vivo* immunogenicity of minigene DNA. In addition, animals such as monkeys having conserved HLA molecules with cross reactivity to CTL and HTL epitopes recognized by human MHC molecules can be used to determine human immunogenicity of HTL and CTL epitopes
5 (Bertoni *et al.*, *J. Immunol.*161:4447-4455 (1998)).

This study represents the first description of the use of HLA transgenic mice to quantitate the *in vivo* immunogenicity of DNA vaccines, by examining response to epitopes restricted by human HLA antigens. *In vivo* studies are required to address the variables crucial for vaccine development, that are not easily evaluated by *in vitro* assays,
10 such as route of administration, vaccine formulation, tissue biodistribution, and involvement of primary and secondary lymphoid organs. Because of its simplicity and flexibility, HLA transgenic mice represent an attractive alternative, at least for initial vaccine development studies, compared to more cumbersome and expensive studies in higher animal species, such as nonhuman primates. The *in vitro* presentation studies
15 described above further supports the use of HLA transgenic mice for screening DNA constructs containing human epitopes inasmuch as a direct correlation between *in vivo* immunogenicity and *in vitro* presentation was observed. Finally, strong CTL responses were observed against all six A 2.1 restricted viral epitopes and in three A11 restricted epitopes encoded in the prototype pMin.1 construct. For five of the A 2.1 restricted
20 epitopes, the magnitude of CTL response approximated that observed with the lipopeptide, Theradigm-HBV, that previously was shown to induce strong CTL responses in humans (Vitiello *et al.*, *J. Clin. Invest.* 95:341 (1995); Livingston *et al.*, *J. Immunol.* 159:1383 (1997)).

Table 1
HBV derived HTL epitopes

Peptide	Sequence	Source	SEQ ID NO:
1298.06	KQAFSPSTYKAFLC	HBV POL 661	137
F107.03	LQSLTNLSSNLSWL	HBV POL 412	138
1280.06	AGFFLLTRILTIQS	HBV ENV 180	139
1280.09	GTSFVYVPSALNPAD	HBV POL 774	140
CF-08	VSEGVWIRTPPYRPPNAPI	HBV NUC 120	141
27.0280	GVWIRTPPYRPPNA	HBV NUC 123	142
1186.25	SPGVWIRTPPYRPP	HBV NUC 121	143
27.0281	RHYLHTLWKAGILYK	HBV POL 145	144
F107.04	PFLLAQFSAICSVV	HBV POL 523	145
1186.15	LVPFVQWFGLSPTV	HBV ENV 339	146
1280.15	LHLYSHPIILGFRKI	HBV POL 501	147
1298.04	KQCFRKL PVNRPIDW	HBV POL 615	148
1298.07	AANWILRGTSFVYVP	HBV POL 764	149
857.02	PHHTALRQATLCWGELMTLA	HBV CORE 50	150
35.0100	LCQVFADATTTGWGL	HBV POL 683	151
35.0096	ESRLVVDPSQFSRGN	HBV POL 387	152
35.0093	VGPLTVNEKRRIKLI	HBV POL 96	153
1186.18	NLSWLSLDVSAAFYH	HBV POL 422	154

Table 2
HIV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO:
A2	924.07	FLPSDFPSV	HIV core 18-27	91
	1013.0102	WLSLLVFFV	HIVadr-ENV (S Ag 335-343)	107
	777.01	FLLRRLTI	HIV ENV aya 183	155
	927.15	ALMPLVACI	HIV aya pol 642	156
	1168.02	GLSRVVARL	HIV POL 455	99
	927.11	FLSLGHIL	HIV pol 562	157
	1147.16	ITLWKAGILYK	HIV POL 149	88
	1083.01	STLPETTVRR	HIV core 141	103
	1090.11	SAICSVVRR	HIV pol 531	158
	1090.10	QAFTFSPTYK	HIV pol 665	159
A3	1069.16	NVSIPWTHK	HIV pol 47	160
	1069.20	LVVDFSQFSR	HIV pol 388	161
	1142.05	KVGNFTGLY	HIV adr POL 629	162
	1069.15	TLWKAGILYK	HIV pol 150	163
	1145.04	IPISSWAF	HIV ENV 313	164
	988.05	LPSDHPSV	HIV core 19-27	165
	1147.04	TPARVTGGVF	HIV POL 354	166
	1069.06	LLVFFVQWFF	HIV env 338-347	167
	1147.13	FLLAQFTSAI	HIV POL 513	168
	1147.14	VLLDYQQMLPV	HIV ENV 259	169
A2	1132.01	LVFFVQWFF	HIV ENV 339	170
	1069.05	FLAQFTSAI	HIV pol 504-512	171
	927.42	NLSWLSIDV	HIV pol 411	172
	927.41	LLSNLSWI	HIV pol 992	173
	927.46	KLHLYSHM	HIV pol 489	174
	1069.071	FLAQFTSA	HIV pol 503	175
	1142.07	GLLGWSIQA	HIV ENV 62	176
	927.47	ILYSIHIL	HIV aya pol 1076	177
	1069.13	PLIPHFCL	HIV env 377-385	178
	1013.1402	VLQAGFILL	HIVadr-ENV 177	179
A3	1090.14	YMDIDVVLGA	HIV pol 538-546	95
	26.0539	RLVVDFSQFSR	HIV pol 376	180
	26.0535	GVWIRTPPAYR	HIV X nuc fas 299	181

Table 2 (Cont'd)
HBV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO:
A3	26.0153	SSAGPCALR	HBV X 64	182
	1.0993	KVFVLGGCR	HBV adr "X" 1548	183
	26.0149	CALRFISAR	HBV X 69	184
	26.0023	VSEGVWIR	HBV x mic fus 296	185
	26.0545	TLPIETTVVRRR	HBV x mic fus 318	186
	20.0111	SVVRRAPTH	HBV POL 524	187
	1.0219	FVLGKCRHK	HBV adr "X" 1550	188
	26.0008	FIFSPTYK	HBV pol 656	189
	20.0130	AFIESPTYK	HBV POL 655	190
	1147.05	FPHCLAFSYM	HBV POL 530	191
B7	1147.08	YPALMPLYA	HBV POL 640	192
	1147.06	LPVCAFFSA	HBV X 58	193
	1147.02	HPAAMPILL	HBV POL 429	194
	26.0570	YPALMPLYACI	HBV pol 640	195
	19.0014	YPALMPLY	HBV POL 640	196
	1145.08	FPHCLAFSY	HBV POL 541	197
Other	1090.02	AYRPPNATH	HBV NUC 131	198
	1.0519	DLIDTASALY	HBV adr CORE 419	199
	13.0129	IFVLVSFGVWI	HBV NUC 117	200
	20.0254	FAAPFTQCGY	HBV POL 631	201
	2.0060	GYPALMPLY	HBV ALL 1224	202
	1069.04	ITLWKAGHLY	HBV pol 149	203
	1069.08	ILLCLIFLL	HBV env 249-258	204
	1.0166	KVGNFTGLY	HBV adr POL 629	162
	1069.23	KYTSFPWLL	HBV POL 745	205
	1069.01	LLDTASALY	HBV core 59	26
	2.0239	ISLDVSAAPY	HBV ALL 1000	207
	2.0181	LYSHIPILGF	HBV POL 492	208
	1039.01	MMWYWGPSLY	HBV 360	209
	2.0126	MSTTDLEAY	HBV adr 1521	210
	1069.03	PIDKGKPY	HBV pol 124	211
	1090.09	PTTGRTSLY	HBV pol 808	212
	20.0138	PWLIKVGNF	HBV POL 51	213
	20.0135	RWMCIRRIH	HBV ENV 236	214
	20.0269	RWMCIRRIH	HBV ENV 236	215
	20.0139	SFCGSPYSW	HBV POL 167	216

Table 2 (Cont'd)
HIV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO:
Other	1069.02	SLDVSAIFY	HIV pol 427	217
	20.0136	SWLSILVPF	HIV ENV 334	218
	20.0271	SWPKFAVPNL	HIV POL 392	219
	20.0137	SWWTSINFL	HIV ENV 197	220
	2.0173	SYQHFRKILL	HIV POL 4	221
	13.0073	WPIHSLTF	HIV NUC 102	222
	1.0774	WLWGMDIDPY	HIV adw CORIS 416	223
	1039.06	WMMWYWGPSLY	HIV env 359	224
	924.14	FLPSDFPFI	HIV 18-27 I ₆ var.	225
	1090.77	YMDDVVLGV	HIV pol 538-546 sub	462
	941.01	FLPSDYFISV	HIV 18-27 analog	226
	1083.02	STLPETYVVR	HIV core 141-151 analog	227
	1145.05	PIIPSSWAF	HIV ENV 313 analog	228
	1145.11	PIICLAFSL	HIV POL 541 analog	229
	1145.24	PIICLAFAL	HIV POL 541 analog	230
	1145.06	PIITSSWAF	HIV ENV 313 analog	231
	1145.23	PIIPMSWAF	HIV ENV 313 analog	232
	1145.07	PIILSSWAF	HIV ENV 313 analog	233
	1145.09	PIVCLAFSY	HIV POL 541 analog	234
	1145.10	PIICLAFAY	HIV POL 541 analog	235

Table 3
HCV derived HTL epitopes

Peptide	Sequence	Source	SEQ ID NO:
P98.03	AAVAAQGYKVLVLPNSVAAATLGFAY	HCV NS3 1242-1267	
P98.04	AAVAAQGYKVLVLPNSVAAAT	HCV NS3 1242	
P98.05	GYKVLVLPNSVAAATLGFAY	HCV NS3 1248	
1283.21	GYKVLVLPNSVAAAT	HCV NS3 1248	
1283.20	GYKVLVLPNSVAAATL	HCV NS3 1253	
	AQGYKVLVLPNSVAA	HCV NS3 1251	
F134.08	GEAVQWMNRLLIAFASRGNHVS	HCV NS4 1914-1935	
1283.44	GEAVQWMNRLLIAFASRGNHIV	HCV NS4 1914	
1283.16	MNRLLIAFASRGNHVS	HCV NS4 1921	
1283.55	SKGWRLLAIPITAYAQ	HCV NS3 1025	
F134.05	GSSYGFQYSPGQRIE	HCV NS5 2641	
1283.61	NFISGIQYLAGLSTLPGNPA	HCV NS4 1772	
1283.25	ASCLRKLGVPPLRVW	HCV NS5 2939	
35.0107	GRHLIFCHSKKCCDE	HCV NS3 1393	
35.0106	TVDFSLDPTFTIET	HCV 1466	
	VVVVATDALMTGYTG	HCV 1437	

Table 4
HCV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO.
A2	1090.18	FLLADARV	HCV NS1/E2 728	
	1073.05	LLPNILGGWV	HCV NS4 1812	
	1013.02	YLVAAYQATV	HCV NS3 1590	
	1013.1002	ILMGGYIPLV	HCV Core 132	
	1090.22	RLVFPDLGV	HCV NS5 2611	
	24.0075	VLVGGVLAA	HCV NS4 1666	
	24.0073	WMNRLIAFA	HCV NS4 1920	
	1174.08	HMWNFISGI	HCV NS4 1769	
	1073.06	ILAGYGAGV	HCV NS4 1851	
	24.0071	LLFLLADA	HCV NS1/E2 726	
	1073.07	YLLPRRGPRL	HCV Core 35	
	1.0119	YLVTRHADV	HCV NS3 1136	
A3	1.0952	KTSESRQPR	HCV Core 51	
	1073.10	GVAGALVAFK	HCV NS4 1863	
	1.0123	LIFCHSKKK	HCV NS3 1391	
	1.0955	QLFTFSPRR	HCV E1 290	
	1073.11	RLGVRATRK	HCV Core 43	
	1073.13	RMYYVGGVEHR	HCV NS1/E2 635	
	24.0090	VAGALVAFK	HCV NS4 1864	
	F104.01	VGIYLLPNR	HCV NS5 3036	
	1145.12	LPGCFSIF	HCV Core 168	
	29.0035	IPFYGKAI	HCV 1378	
B7	1069.62	CTCGSSDLY	HCV NS3 1128	
	24.0092	FWAKIMWNF	HCV NS4 1765	
	13.0019	LSAFSLHSY	HCV NS5 2922	
Other				

WO 99/58658

Table 4 (Cont'd)
HCV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO.
A3	24.0086	LGFGAYMSK	HCV NS3 1267	
	1174.21	RVCEKMAALY	HCV NS5 2621	
	1174.16	WMNSTGFTK	HCV NS1/E2 557	
	1073.04	TLIGTPIILY	HCV NS3 1622	
	16.0012	PPYLVAAYQA	HCV NS3 1588	
B7	15.0047	YPCIVNFTI	HCV NS1/E2 623	
	24.0093	EVDGVRILIRY	HCV NS5 2129	
Other	3.0417	LTCGFADLMGY	HCV 126	
	1073.01	NIVDVQYLY	HCV E1 700	
	1.0509	GLSAFSLIISY	HCV NS5 2921	
	1073.17	MYVGDLCGSVF	HCV E1 275	
	1073.18	MYVGGVEHRL	HCV NS1/E2 633	
	13.075	QYLAGLSTL	HCV NS4 1778	
	1145.13	HPGCSFSIF	HCV Core 168	
	1145.25	LPGCMFSIF	HCV Core 168	
	1292.24	LPGCSFSII	HCV Core 169	
	1145.14	LPVCSFSIF	HCV Core 168	
	1145.15	LPGCSFSYF	HCV Core 168	

Table 5
HIV derived HTL epitopes

Peptide	Sequence	Source	SEQ ID NO:
27.0313	GEYKRWIILGLNKIVRMYSPTSILD	HIV GAG 294-319	
27.0311	KRWIILGLNKIVRMYSPTSILD	HIV gag 298-319	
27.0354	KRWIILGLNKIVRMYS	HIV GAG 298	
27.0377	GEYKRWIILGLNKI	HIV GAG 294	
	WEFVNTPLVLKWLWYQ	HIV POL 596	
	QKQITKIQNFRVYYR	HIV POL 956	
1280.03	EKVYLA WVP AHKGIGG	HIV POL 711-726	
27.0361	KVYLA WVP AHKGIGG	HIV POL 712	
	EKVYLA WVP AHKGIG	HIV POL 711	
27.0304	PIVQNIQGMVHQAI SPRTLNA	HIV gag 165-186	
27.0297	QGMVHQAI SPRTL N	HIV GAG 171	
27.0344	QHLLQLTVWG IKQLQ	HIV ENV 729	
F091.15	SPAIFQSSMTKILEP	HIV POL 335	
27.0341	IKQFINMWQEVGKAMY	HIV ENV 566	
27.0364	FRKYTAFTIPSINNE	HIV POL 303	
27.0373	HSNWRAMASDFNLPP	HIV POL 758	
	KTAVQMAVEIHNEKR	HIV POL 915	
	DRVHPVHAGPIAPGQMREPRGS	HIV GAG 245	
	AFSPEVPMFSALSEGA TPQDLNTML	HIV gag 195-216	
	AFSPEVPMFSALSEGA TPQDL	HIV gag 195-216	
200.06	SALSEGA TPQDLNTML	HIV gag 205	
27.0307	SPEVPMFSALSEGA	HIV gag 197	
	I.QEQIGWM'TNNP'IPVGEIYKR	HIV gag 275	
27.0310	QEQIGWM'TNNP'IPV	HIV gag 276	
35.0135	YRKILRQRKIDRLID	HIV VPU 31	
35.0131	WAGIKQEFGIPYNPQ	HIV POL 874	
35.0127	EVNIV'IDSQYALGI	HIV POL 674	
35.0125	AETFYVDGAANRET K	HIV POL 619	
35.0133	GAVVIQDNSDIKVVP	HIV POL 989	

Table 6
HIV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO:
A2	25.0148	MASDFNLPPV	HIV1 POL 70	
	1069.32	VLAZAMSQV	HIV gag 397	
	1211.04	KLTPLCVTL	HIV ENV 134	
	25.0062	KLVGKLNWA	HIV1 POL 87	
	25.0039	LTFGWCFKL	HIV1 NEF 62	
	941.031	ILKEPVHGV	HIV1 pol 476-484	
	25.0035	MTNNPPIPV	HIV1 GAG 34	
	25.0057	RILQQLLFI	HIV1 VPR 72	
	1.0944	AVEIHNFKR	HIV POL 1434	
	1.1056	KIQNFRVYYR	HIV POL 1474	
A3	1069.49	QMAVFIHNFK	HIV pol 1432	
	966.0102	AIFQSSMTK	HIV pol 337	
	1150.14	MAVFIHNFK	HIV pol 909	
	940.03	QVPLRPMTYK	HIV nef 73-82	
	25.0175	TTLFCASDAK	HIV1 ENV 81	
	1069.43	TVYYGVVPVWK	HIV env 49	
	25.0209	VTIKIGGQLK	HIV1 POL 65	
	1146.01	FPVRPQVPL	HIV nef 84-92	
	29.0060	IPIHYCAPA	HIV env 293	
	15.0073	FPISPIETV	HIV POL 171	
B7	29.0056	CPKVSFEPI	HIV env 285	
	29.0107	IPYNPQSQQVV	HIV pol 883	
	25.0151	CTLNFPISPI	HIV1 POL 96	
	25.0143	LTPGWCFKLV	HIV1 NEF 62	
	25.0043	YTAFTIPSI	HIV1 POL 83	
	25.0055	AIRILQQL	HIV1 VPR 76	
	25.0049	ALVEICTEM	HIV1 POL 52	
	25.0032	LIQLTVWGI	HIV1 ENV 61	
	25.0050	LVGPTPVNI	HIV1 POL 100	
	25.0047	KAACWWAGI	HIV1 POL 65	
A2	25.0162	KMIGGIGGI	HIV1 POL 96	
	25.0052	RAMASDFNL	HIV1 POL 78	
	1211.09	SLLNATDIAV	HIV ENV 814	

Table 6 (Cont'd)
HIV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO:
A2	25.0041	TLNFPISPI	HIV1 POL 96	
A3	1.0046	IVIWGKTPK	HIV POL 1075	
	25.0064	MVHQAI SPR	HIV1 GAG 45	
	1.0062	YLA WVP AIK	HIV POL 1227	
	1.0942	MTKILEPFR	HIV POL 859	
	25.0184	QMVHQAI SPR	HIV1 GAG 45	
	1069.48	AVFHINEK RK	HIV pol 1434	
	1069.44	KLAGRW P VK	HIV pol 1358	
	1069.42	KVYLA WVP AHK	HIV pol 1225	
	1.0024	NTPVFAIKK	HIV pol 752	
	25.0062	RIVELIGRR	HIV1 ENV 53	
	25.0095	TIKIGGQ LK	HIV1 POL 65	
	25.0078	TLFCASDAK	HIV1 ENV 82	
	25.0104	VMIVWQVDR	HIV1 VIF 83	
	1069.47	VTVYYGV P VK	HIV env 48	
B7	15.0268	YPLASLRSLF	HIV GAG 507	
	1292.13	HPVHAGPIA	HIV GAG 248	
	19.0044	VPLQLPPL	HIV con. REV 71	
Other	1.0431	EVNIVTDSQY	HIV POL 1187	
	1.0014	FRDYVDRFY	HIV GAG 298	
	25.0113	IWGCSGKI I	HIV1 ENV 69	
	25.0127	IYETYGDTW	HIV1 VPR 92	
	1069.60	IYQEPFKNL	HIV pol 1036	
	2.0129	IYQYMDDL Y	HIV pol 359	
	25.0128	PYNEWTLHL	HIV1 VPR 56	
	25.0123	PYNTTPVFAI	HIV1 POL 74	
	1069.57	RYLKDQQLL	HIV env 2778	
	1069.58	RYLRDQQLL	HIV env 2778	
	1069.59	TYQIYQEPF	HIV pol 1033	
	1069.27	VYQYMDL DLY	HIV pol 358	
	1069.26	VTVLDVGDAY	HIV pol 265	
	25.0115	VWKEATTIL	HIV1 ENV 47	
	25.0218	VWKEATTILF	HIV1 ENV 47	
	25.0219	YMQATWIPEW	HIV1 POL 96	

Table 6 (Cont'd)
HIV derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO.
A2	1211.4	SLLNATAIAV	HIV MN gp160 814(a)	
A3	F105.21	AIFQRSMTR	HIV pol 337(a)	
	F105.17	AIFQSSMTR	HIV pol 337(a)	
	F105.02	GHFQSSMTK	HIV pol 337(a)	
	F105.03	AAIFQSSMTK	HIV pol 337(a)	
	F105.04	AIAQSSMTK	HIV pol 337(a)	
	F105.05	AIFASSMTK	HIV pol 337(a)	
	F105.06	AIFQASMTK	HIV pol 337(a)	
	F105.07	AIFQSAMTK	HIV pol 337(a)	
	F105.08	AIFQSSATK	HIV pol 337(a)	
	F105.09	AIFQSSMAK	HIV pol 337(a)	
	F105.11	FIFQSSMTK	HIV pol 337(a)	
	F105.12	SIFQSSMTK	HIV pol 337(a)	
	F105.16	AIFQCSMTK	HIV pol 337(a)	
B7	1145.03	FPVRPQFPL	HIV nef 84-92 analog	
	1181.03	FPVRPQVPI	HIV nef 84-92(a)	
	1292.14	HPVHAGPII	HIV GAG 248	
	1292.09	FPISPIETI	HIV POL 179	
	1145.02	FPVTPQVPL	HIV nef 84-92 analog	
	1145.22	FPVRMQVPL	HIV nef 84-92 analog	
	1181.04	FPVRPQVPM	HIV nef 84-92(a)	
	1181.01	FPVRPQVPA	HIV nef 84-92(a)	
	1181.02	FPVRPQVPV	HIV nef 84-92(a)	
	1181.05	FPVRPQVPF	HIV nef 84-92(a)	
	1181.06	FPVRPQVPW	HIV nef 84-92(a)	

Table 7
P. falciparum derived H'VL epitopes

Peptide	Sequence	Source	SEQ ID NO:
F125.04	RINWVNIIHAVPLAMKLI	Pf SSP2 61	
1188.34	IINWVNIIHAVPLAMKLI	Pf SSP2 62	
1188.16	KSKYKLATSVLAGLI	Pf EXP1 71	
	LVNLLIFIIHNGKIIKNS	Pf LSA1 13	
F125.02	LVNLLIFHNGKIIKNS	Pf LSA1 13	
27.0402	LLIFHNGKIIKNS	Pf LSA1 16	
1188.32	GLAYKFVVPGAATPY	Pf SSP2 512	
27.0392	SSVFNVVNSSIGLIM	Pf CSP 410	
27.0417	VKNVIGPFMKAVCVE	Pf SSP2 223	
27.0388	MRKLAILSVSSFLFV	Pf CSP 2	
27.0387	MNYYGKQENWYSLKK	Pf CSP 53	
1188.38	KYKIAGGIAGGLALL	Pf SSP2 494	
1188.13	AGLLGNVSTVLLGGV	Pf EXP1 82	
27.0408	QTNFKSLRLNLGVSE	Pf LSA1 94	
35.0171	PDSIQDSLKESRKLN	Pf SSP2 165	
35.0172	KCNLYADSAWENVKN	Pf SSP2 211	

Table 8
P. falciparum derived CTL epitopes

Supertype	Peptide	Sequence	Source	SEQ ID NO:
A2	1167.21	FLIFFDLFLV	Pf SSP2 14	
	1167.08	GLIMVLSFL	Pf CSP 425	
	1167.12	VLAGLIGNV	Pf EXP1 80	
	1167.13	KILSVFFLA	Pf EXP1 2	
	1167.10	GLLGNVSTV	Pf EXP1 83	
	1167.18	ILSVSSFLV	Pf CSP 7	
	1167.19	VLLGGVGLVL	Pf EXP1 91	
A3	1167.36	LACAGLAYK	Pf SSP2 511	
	1167.32	QTNFKSLIR	Pf LSA1 94	
	1167.43	VTCGNGIQVR	Pf CSP 375	
	1167.24	ALFFIIFNK	Pf EXP1 10	
	1167.28	GVSENIIFLK	Pf LSA1 105	
	1167.47	IIVLSHNSYEK	Pf LSA1 59	
	1167.51	LLACAGLAYK	Pf SSP2 510	
	1167.46	FILVNLLIHI	Pf LSA1 11	
	1101.03	MPLTQLAI	Pf SHEBA 77	
	1167.61	TPYAGEPAPF	Pf SSP2 539	
	1167.14	FLIFFDLFL	Pf SSP2 14	
A2	1167.16	FMKAVCQEV	Pf SSP2 230	
	1167.15	LIFFDLFLV	Pf SSP2 15	
	1167.17	LLMDCSGSI	Pf SSP2 51	
	1167.09	VLLGGVGLV	Pf EXP1 91	
	19.0051	LPYGRINI	Pf SSP2 126	
Other	16.0245	PQDEENIGY	Pf LSA1 1794	
	16.0040	FVEALFQEY	Pf CSP 15	
	1167.54	FYFILVNLL	Pf LSA1 9	
	1167.53	KYKLATSVL	Pf EXP1 73	
	1167.56	KYLVIVFLI	Pf SSP2 8	
	15.0184	LPSENERGY	Pf LSA1 1663	
	16.0130	PSDGKCNLY	Pf SSP2 207	
	16.0077	PSENERGY	Pf LSA1 1664	
	1167.57	PYAGEPAPF	Pf SSP2 528	
	1167.55	YYIPHQSSL	Pf LSA1 1671	

Table 9. Activation of T Cell Proliferation by Expression
 Vectors Encoding MHC Class II Epitopes Fused to MHC
 Class II Targeting Sequences

5

	Immunogen	Stimulating Peptide ¹		
		PADRE	OVA 323	CORE 128
	peptide – CFA ²	3.0 (1.1)	2.7 (1.2)	3.2 (1.4)
10	pEP2.(PAOS).(-)	-	-	-
	pEP2.(AOS).(-)	5.6 (1.8)	-	-
	pEP2.(PAOS).(sigTh)	5.0 (2.9)	-	2.6 (1.5)
	pEP2.(PAOS).(Ig α Th)	5.6 (2.1)	-	3.0 (1.6)
	pEP2.(PAOS).(LampTh)	3.8 (1.7)	-	3
15	pEP2.(PAOS).(IiTh)	5.2 (2.0)	3.2 (1.5)	3.7 (1.5)
	pEP2.(PAOS).(H2M)	3.3 (1.3)	-	2.8

¹Geometric mean of cultures with SI $\geq$ 2.

²Proliferative response measured in the lymph node.

20

Table 10
CTL Epitopes in cDNA Minigene

Epitope	Sequence	MHC Restrict.	MHC Binding Affinity [IC30% (nM)]	Immunogenicity In Vivo (IFA)	
				No. CTL- Positive Cultures	CTL Response (Geo. Mean x/±SD) ^b ΔLU
HBV Core 18	FLPSDFFPSV	A2.1	3	6 / 6	73.0 (1.1)
HBV Env 335	WLSLLVPFV	A2.1	5	4 / 6	5.3 (1.6)
HBV Pol 455	GLSRYVARL	A2.1	76	ND ^c	ND
HIV Env 120	KLTPLCVTL	A2.1	102	2 / 5	6.4 (1.3)
HIV Pol 476	ILKEPVHGV	A2.1	192	2 / 5	15.2 (2.9)
HBV Pol 551-A	YMDDVVLGA	A2.1	200	0 / 6	-
HBV Pol 551-V	YMDDVVLGV	A2.1	5	6 / 6	8.2 (2.3)
HIV Env 49	TVYYGVPVWK	A11	4	28 / 33	13.4 (3.1)
HBV Core 141	STLPETT VRR	A11	4	6 / 6	12.1 (2.6)
HBV Pol 149	HTLWKAGILYK	A11	14	6 / 6	13.1 (1.2)

a Peptide tested in HLA-A2.1/K^b H-2^{bbs} transgenic mice by co-immunizing with a T helper cell peptide in IFA.

5 b Geometric mean CTL response of positive cultures.

c ND, not done.

Table 11
Summary of Immunogenicity of pMin.1 DNA
construct in HLA A2.1/K^b transgenic mice

Epitope	CTL Response ^a	
	No. Positive Cultures/Total ^b	Geo. Mean Response Positive Cultures [x/±SD] ΔLU
HBV Core 18	9 / 9	455.5 [2.2]
HIV Env 120	12 / 12	211.9 [3.7]
HBV Pol 551-V	9 / 9	126.1 [2.8]
HBV Pol 455	12 / 12	738.6 [1.3]
HIV Pol 476	11 / 11	716.7 [1.5]
HBV Env 335	12 / 12	43.7 [1.8]
HBV Core 18 (Theradigm) ^c	10 / 10	349.3 [1.8]

^a Mice were immunized with pMin.1 DNA or Theradigm-HBV lipopeptide and CTL activity in splenocyte cultures was determined after in vitro stimulation with individual peptide epitopes. Results from four independent experiments are shown.

^b See Example V, Materials and Methods for definition of a CTL-positive culture.

^c Response of mice immunized with Theradigm-HBV lipopeptide containing the HBV Core 18 epitope.

Table 12
Summary of immunogenicity
in HLA A11/K^b transgenic mice

Epitope	CTL Response ^a	
	No. Positive Cultures/Total ^b	Geo. Mean Response Positive Cultures [$\bar{x}$ $\pm$ SD]
		Δ LU
HBV Core 141	5/9	128.1 [1.6]
HBV Pol 149	6/9	267.1 [2.2]
HIV Env 43	9/9	40.1 [2.9]

^a Mice were immunized with pMin.1 DNA and CTL activity in splenocyte cultures was determined after in vitro stimulation with individual A11-restricted epitopes. The geometric mean CTL response from three independent experiments are shown.

^b Definition of a CTL-positive culture is described in Example V, Materials and Methods.

SEQUENCE LISTING

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<130> 1987-126div

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II epitopes

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cta gac aag ctg acc atc acc tcc cag aac ctg caa ctg gag agc ctt      243
Leu Asp Lys Leu Thr Ile Thr Ser Gln Asn Leu Gln Leu Glu Ser Leu
      65                      70                      75

cgc atg aag ctt atc agc cag gct gtg cac gcc gct cac gcc gaa atc      291
Arg Met Lys Leu Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile
      80                      85                      90

```

aac gaa gct gga aga acc cct cca gct tat cgc cct cca aac gct cct 339
 Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro
 95 100 105

atc ctg ttc ttt ctg ctg acc aga atc ctg aca atc ccc cag tcc ctg 387
 Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu
 110 115 120

gac gcc aag ttc gtg gct gcc tgg acc ctg aag gct gcc gct 429
 Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
 125 130 135

tgaggtacc 438

<210> 4
 <211> 138
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> I80T

<400> 4
 Met Asp Asp Gln Arg Asp Leu Ile Ser Asn His Glu Gln Leu Pro Ile
 1 5 10 15
 Leu Gly Asn Arg Pro Arg Glu Pro Glu Arg Cys Ser Arg Gly Ala Leu
 20 25 30
 Tyr Thr Gly Val Ser Val Leu Val Ala Leu Leu Leu Ala Gly Gln Ala
 35 40 45
 Thr Thr Ala Tyr Phe Leu Tyr Gln Gln Gln Gly Arg Leu Asp Lys Leu
 50 55 60
 Thr Ile Thr Ser Gln Asn Leu Gln Leu Glu Ser Leu Arg Met Lys Leu
 65 70 75 80
 Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
 85 90 95
 Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe
 100 105 110
 Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe
 115 120 125
 Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
 130 135

<210> 5
 <211> 783
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> IiThfull construct encoding fusion of the
 cytoplasmic, transmembrane and part of the luminal
 domains of the murine Ii protein gene fused to
 multiple T helper epitopes and amino acid residues
 101-215 trimerization region of the Ii protein

<221> CDS
 <222> (16)...(777)
 <223> IiThful

<400> 5

gctagcgccg ccacc atg gat gac caa cgc gac ctc atc tct aac cat gag	51
Met Asp Asp Gln Arg Asp Leu Ile Ser Asn His Glu	
1 5 10	
caa ttg ccc ata ctg ggc aac cgc cct aga gag cca gaa agg tgc agc	99
Gln Leu Pro Ile Leu Gly Asn Arg Pro Arg Glu Pro Glu Arg Cys Ser	
15 20 25	
cgt gga gct ctg tac acc ggt gtt tct gtc ctg gtg gct ctg ctc ttg	147
Arg Gly Ala Leu Tyr Thr Gly Val Ser Val Leu Val Ala Leu Leu Leu	
30 35 40	
gct ggg cag gcc acc act gct tac ttc ctg tac cag caa cag ggc cgc	195
Ala Gly Gln Ala Thr Thr Ala Tyr Phe Leu Tyr Gln Gln Gln Gly Arg	
45 50 55 60	
cta gac aag ctg acc atc acc tcc cag aac ctg caa ctg gag agc ctt	243
Leu Asp Lys Leu Thr Ile Thr Ser Gln Asn Leu Gln Leu Glu Ser Leu	
65 70 75	
cgc atg aag ctt atc agc cag gct gtg cac gcc gct cac gcc gaa atc	291
Arg Met Lys Leu Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile	
80 85 90	
aac gaa gct gga aga acc cct cca gct tat cgc cct cca aac gct cct	339
Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro	
95 100 105	
atc ctg ttc ttt ctg ctg acc aga atc ctg aca atc ccc cag tcc ctg	387
Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu	
110 115 120	
gac gcc aag ttc gtg gct gcc tgg acc ctg aag gct gcc gct atg tcc	435
Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Met Ser	
125 130 135 140	
atg gat aac atg ctc ctt ggg cct gtg aag aac gtt acc aag tac ggc	483
Met Asp Asn Met Leu Leu Gly Pro Val Lys Asn Val Thr Lys Tyr Gly	
145 150 155	
aac atg acc cag gac cat gtg atg cat ctg ctc acg agg tct gga ccc	531
Asn Met Thr Gln Asp His Val Met His Leu Leu Thr Arg Ser Gly Pro	
160 165 170	
ctg gag tac ccg cag ctg aag ggg acc ttc cca gag aat ctg aag cat	579
Leu Glu Tyr Pro Gln Leu Lys Gly Thr Phe Pro Glu Asn Leu Lys His	
175 180 185	
ctt aag aac tcc atg gat ggc gtg aac tgg aag atc ttc gag agc tgg	627
Leu Lys Asn Ser Met Asp Gly Val Asn Trp Lys Ile Phe Glu Ser Trp	
190 195 200	
atg aag cag tgg ctc ttg ttt gag atg agc aag aac tcc ctg gag gag	675
Met Lys Gln Trp Leu Leu Phe Glu Met Ser Lys Asn Ser Leu Glu Glu	
205 210 215 220	
aag aag ccc acc gag gct cca cct aaa gag cca ctg gac atg gaa gac	723
Lys Lys Pro Thr Glu Ala Pro Pro Lys Glu Pro Leu Asp Met Glu Asp	
225 230 235	

cta tct tct ggc ctg gga gtg acc agg cag gaa ctg ggt caa gtc acc 771
 Leu Ser Ser Gly Leu Gly Val Thr Arg Gln Glu Leu Gly Gln Val Thr
 240 245 250

ctg tgaggtacc 783
 Leu

<210> 6
 <211> 253
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> IiThfull

<400> 6
 Met Asp Asp Gln Arg Asp Leu Ile Ser Asn His Glu Gln Leu Pro Ile
 1 5 10 15
 Leu Gly Asn Arg Pro Arg Glu Pro Glu Arg Cys Ser Arg Gly Ala Leu
 20 25 30
 Tyr Thr Gly Val Ser Val Leu Val Ala Leu Leu Leu Ala Gly Gln Ala
 35 40 45
 Thr Thr Ala Tyr Phe Leu Tyr Gln Gln Gln Gly Arg Leu Asp Lys Leu
 50 55 60
 Thr Ile Thr Ser Gln Asn Leu Gln Leu Glu Ser Leu Arg Met Lys Leu
 65 70 75 80
 Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
 85 90 95
 Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe
 100 105 110
 Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe
 115 120 125
 Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Met Ser Met Asp Asn Met
 130 135 140
 Leu Leu Gly Pro Val Lys Asn Val Thr Lys Tyr Gly Asn Met Thr Gln
 145 150 155 160
 Asp His Val Met His Leu Leu Thr Arg Ser Gly Pro Leu Glu Tyr Pro
 165 170 175
 Gln Leu Lys Gly Thr Phe Pro Glu Asn Leu Lys His Leu Lys Asn Ser
 180 185 190
 Met Asp Gly Val Asn Trp Lys Ile Phe Glu Ser Trp Met Lys Gln Trp
 195 200 205
 Leu Leu Phe Glu Met Ser Lys Asn Ser Leu Glu Glu Lys Lys Pro Thr
 210 215 220
 Glu Ala Pro Pro Lys Glu Pro Leu Asp Met Glu Asp Leu Ser Ser Gly
 225 230 235 240
 Leu Gly Val Thr Arg Gln Glu Leu Gly Gln Val Thr Leu
 245 250

<210> 7
 <211> 378
 <212> DNA
 <213> Artificial Sequence

<220>

<223> KappaLAMP-Th construct encoding fusion of murine immunoglobulin kappa signal sequence fused to multiple T helper epitopes and the cytoplasmic and transmembrane domains of human lysosomal membrane glycoprotein-1 (LAMP-1)

<221> CDS

<222> (16)...(372)

<223> KappaLAMP-Th

<400> 7

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gctagcgccg ccacc atg gga atg cag gtg cag atc cag agc ctg ttt ctg      51
      Met Gly Met Gln Val Gln Ile Gln Ser Leu Phe Leu
          1              5              10

ctc ctc ctg tgg gtg ccc ggg tcc aga gga atc agc cag gct gtg cac      99
Leu Leu Leu Trp Val Pro Gly Ser Arg Gly Ile Ser Gln Ala Val His
      15              20              25

gcc gct cac gcc gaa atc aac gaa gct gga aga acc cct cca gct tat      147
Ala Ala His Ala Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr
      30              35              40

cgc cct cca aac gct cct atc ctg ttc ttt ctg ctg acc aga atc ctg      195
Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu
      45              50              55              60

aca atc ccc cag tcc ctg gac gcc aag ttc gtg gct gcc tgg acc ctg      243
Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu
          65              70              75

aag gct gcc gct aac aac atg ttg atc ccc att gct gtg ggc ggt gcc      291
Lys Ala Ala Ala Asn Asn Met Leu Ile Pro Ile Ala Val Gly Gly Ala
          80              85              90

ctg gca ggg ctg gtc ctc atc gtc ctc att gcc tac ctc att ggc agg      339
Leu Ala Gly Leu Val Leu Ile Val Leu Ile Ala Tyr Leu Ile Gly Arg
          95              100             105

aag agg agt cac gcc ggc tat cag acc atc taggggtacc      378
Lys Arg Ser His Ala Gly Tyr Gln Thr Ile
      110              115

```

<210> 8

<211> 118

<212> PRT

<213> Artificial Sequence

<220>

<223> KappaLAMP-Th

<400> 8

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Met Gly Met Gln Val Gln Ile Gln Ser Leu Phe Leu Leu Leu Leu Trp
  1              5              10              15
Val Pro Gly Ser Arg Gly Ile Ser Gln Ala Val His Ala Ala His Ala
      20              25              30

```

Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn
 35 40 45

Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln
 50 55 60
 Ser Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
 65 70 75 80
 Asn Asn Met Leu Ile Pro Ile Ala Val Gly Gly Ala Leu Ala Gly Leu
 85 90 95
 Val Leu Ile Val Leu Ile Ala Tyr Leu Ile Gly Arg Lys Arg Ser His
 100 105 110
 Ala Gly Tyr Gln Thr Ile
 115

<210> 9
 <211> 381
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> H2M-Th construct encoding fusion of signal
 sequence of H2-M fused to multiple MHC class II
 epitopes and the cytoplasmic and transmembrane
 domains of H2-M

<221> CDS
 <222> (16)...(375)
 <223> H2M-Th

<400> 9
 gctagcgccg ccacc atg gct gca ctc tgg ctg ctg ctg ctg gtc ctc agt 51
 Met Ala Ala Leu Trp Leu Leu Leu Leu Val Leu Ser
 1 5 10
 ctg cac tgt atg ggg atc agc cag gct gtg cac gcc gct cac gcc gaa 99
 Leu His Cys Met Gly Ile Ser Gln Ala Val His Ala Ala His Ala Glu
 15 20 25
 atc aac gaa gct gga aga acc cct cca gct tat cgc cct cca aac gct 147
 Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala
 30 35 40
 cct atc ctg ttc ttt ctg ctg acc aga atc ctg aca atc ccc cag tcc 195
 Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
 45 50 55 60
 ctg gac gcc aag ttc gtg gct gcc tgg acc ctg aag gct gcc gct aag 243
 Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Lys
 65 70 75
 gtc tct gtg tct gca gcc acc ctg ggc ctg ggc ttc atc atc ttc tgt 291
 Val Ser Val Ser Ala Ala Thr Leu Gly Leu Gly Phe Ile Ile Phe Cys
 80 85 90
 gtt ggc ttc ttc aga tgg cgc aag tct cat tcc tcc agc tac act cct 339
 Val Gly Phe Phe Arg Trp Arg Lys Ser His Ser Ser Ser Tyr Thr Pro
 95 100 105

ctc cct gga tcc acc tac cca gaa gga cgg cat taggggtacc
 Leu Pro Gly Ser Thr Tyr Pro Glu Gly Arg His
 110 115

381

<210> 10
 <211> 119
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> H2M-Th

<400> 10
 Met Ala Ala Leu Trp Leu Leu Leu Leu Val Leu Ser Leu His Cys Met
 1 5 10 15
 Gly Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala
 20 25 30
 Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe
 35 40 45
 Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ala Lys
 50 55 60
 Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Lys Val Ser Val Ser
 65 70 75 80
 Ala Ala Thr Leu Gly Leu Gly Phe Ile Ile Phe Cys Val Gly Phe Phe
 85 90 95
 Arg Trp Arg Lys Ser His Ser Ser Ser Tyr Thr Pro Leu Pro Gly Ser
 100 105 110
 Thr Tyr Pro Glu Gly Arg His
 115

<210> 11
 <211> 432
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> H2O-Th construct encoding fusion of signal
 sequence of H2-DO fused to multiple MHC class II
 epitopes and the cytoplasmic and transmembrane
 domains of H2-DO

<221> CDS
 <222> (16)...(426)
 <223> H2O-Th

<400> 11
 gctagcgccg ccacc atg ggc gct ggg agg gcc ccc tgg gtg gtg gct ctg 51
 Met Gly Ala Gly Arg Ala Pro Trp Val Val Ala Leu
 1 5 10
 ttg gtg aac ctc atg agg ctg gat tcc atc agc cag gct gtg cac gcc 99
 Leu Val Asn Leu Met Arg Leu Asp Ser Ile Ser Gln Ala Val His Ala
 15 20 25
 gct cac gcc gaa atc aac gaa gct gga aga acc cct cca gct tat cgc 147
 Ala His Ala Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg
 30 35 40

cct cca aac gct cct atc ctg ttc ttt ctg ctg acc aga atc ctg aca 195
 Pro Pro Asn Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr
 45 50 55 60
 atc ccc cag tcc ctg gac gcc aag ttc gtg gct gcc tgg acc ctg aag 243
 Ile Pro Gln Ser Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys
 65 70 75
 gct gcc gct ata ctg agt gga gct gca gtg ttc ctg ctt ggg ctg att 291
 Ala Ala Ala Ile Leu Ser Gly Ala Ala Val Phe Leu Leu Gly Leu Ile
 80 85 90
 gtc ttc ctg gtg ggg gtt gtt atc cat ctc aag gct cag aaa gca tct 339
 Val Phe Leu Val Gly Val Val Ile His Leu Lys Ala Gln Lys Ala Ser
 95 100 105
 gtg gag act cag cct ggc aat gag agt agg tcc cgg atg atg gag cgg 387
 Val Glu Thr Gln Pro Gly Asn Glu Ser Arg Ser Arg Met Met Glu Arg
 110 115 120
 cta acc aag ttc aag gct gga ccg gga cat gtc aca tgaggtacc 432
 Leu Thr Lys Phe Lys Ala Gly Pro Gly His Val Thr
 125 130 135

<210> 12
 <211> 136
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> H2O-Th

<400> 12
 Met Gly Ala Gly Arg Ala Pro Trp Val Val Ala Leu Leu Val Asn Leu
 1 5 10 15
 Met Arg Leu Asp Ser Ile Ser Gln Ala Val His Ala Ala His Ala Glu
 20 25 30
 Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala
 35 40 45
 Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
 50 55 60
 Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Ile
 65 70 75 80
 Leu Ser Gly Ala Ala Val Phe Leu Leu Gly Leu Ile Val Phe Leu Val
 85 90 95
 Gly Val Val Ile His Leu Lys Ala Gln Lys Ala Ser Val Glu Thr Gln
 100 105 110
 Pro Gly Asn Glu Ser Arg Ser Arg Met Met Glu Arg Leu Thr Lys Phe
 115 120 125
 Lys Ala Gly Pro Gly His Val Thr
 130 135

<210> 13
 <211> 816
 <212> DNA
 <213> Artificial Sequence

<220>

<223> PADRE-Influenza matrix construct encoding fusion
of pan DR epitope sequence to amino-terminus of
influenza matrix protein gene

<221> CDS

<222> (16)...(816)

<223> PADRE-Influenza matrix

<400> 13

gctagcgccg ccacc atg gcc aag ttc gtg gct gcc tgg acc ctg aag gct	51
Met Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala	
1 5 10	
gcc gct atg agt ctt cta acc gag gtc gaa acg tac gtt ctc tct atc	99
Ala Ala Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile	
15 20 25	
atc cca tca ggc ccc ctc aaa gcc gag atc gcg cag aga ctt gag gat	147
Ile Pro Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp	
30 35 40	
gtt ttt gca ggg aag aac aca gat ctt gag gct ctc atg gaa tgg cta	195
Val Phe Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu	
45 50 55 60	
aag aca aga cca atc ctg tca cct ctg act aag gga att tta ggg ttt	243
Lys Thr Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe	
65 70 75	
gtg ttc acg ctc acc gtg ccc agt gag cga gga ctg cag cgt aga cga	291
Val Phe Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg	
80 85 90	
ttt gtc caa aat gcc cta aat ggg aat gga gac cca aac aac atg gac	339
Phe Val Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp	
95 100 105	
agg gca gtt aaa cta tac aag aag ctg aag agg gaa atg aca ttc cat	387
Arg Ala Val Lys Leu Tyr Lys Lys Leu Lys Arg Glu Met Thr Phe His	
110 115 120	
gga gca aag gaa gtt gca ctc agt tac tca act ggt gcg ctt gcc agt	435
Gly Ala Lys Glu Val Ala Leu Ser Tyr Ser Thr Gly Ala Leu Ala Ser	
125 130 135 140	
tgc atg ggt ctc ata tac aac cgg atg gga aca gtg acc aca gaa gtg	483
Cys Met Gly Leu Ile Tyr Asn Arg Met Gly Thr Val Thr Thr Glu Val	
145 150 155	
gct ctt ggc cta gta tgt gcc act tgt gag cag att gct gat gcc caa	531
Ala Leu Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ala Gln	
160 165 170	
cat cgg tcc cac agg cag atg gcg act acc acc aac cca cta atc agg	579
His Arg Ser His Arg Gln Met Ala Thr Thr Thr Asn Pro Leu Ile Arg	
175 180 185	

```

cat gag aac aga atg gta cta gcc agc act acg gct aag gcc atg gag      627
His Glu Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu
    190                      195                      200

caa atg gct gga tca agt gag cag gca gca gag gcc atg gaa gtc gca      675
Gln Met Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala
    205                      210                      215                      220

agt cag gct aga caa atg gtg cag gca atg agg aca att ggg act cac      723
Ser Gln Ala Arg Gln Met Val Gln Ala Met Arg Thr Ile Gly Thr His
                      225                      230                      235

cct agc tcc agt gca ggt cta aaa gat gat ctt att gaa aat ttg cag      771
Pro Ser Ser Ser Ala Gly Leu Lys Asp Asp Leu Ile Glu Asn Leu Gln
                      240                      245                      250

gct tac cag aaa cgg atg ggg gtg cag atg cag cga ttc aag      813
Ala Tyr Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
    255                      260                      265

tga                                                                    816

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<210> 14
<211> 266
<212> PRT
<213> Artificial Sequence

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```

<220>
<223> PADRE-Influenza matrix

```

```

<400> 14
Met Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Met Ser
 1                      5                      10                      15
Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro Ser Gly
    20                      25                      30
Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe Ala Gly
    35                      40                      45
Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr Arg Pro
    50                      55                      60
Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe Thr Leu
    65                      70                      75                      80
Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val Gln Asn
    85                      90                      95
Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Arg Ala Val Lys
    100                      105                      110
Leu Tyr Lys Lys Leu Lys Arg Glu Met Thr Phe His Gly Ala Lys Glu
    115                      120                      125
Val Ala Leu Ser Tyr Ser Thr Gly Ala Leu Ala Ser Cys Met Gly Leu
    130                      135                      140
Ile Tyr Asn Arg Met Gly Thr Val Thr Thr Glu Val Ala Leu Gly Leu
    145                      150                      155                      160
Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ala Gln His Arg Ser His
    165                      170                      175
Arg Gln Met Ala Thr Thr Thr Asn Pro Leu Ile Arg His Glu Asn Arg
    180                      185                      190
Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met Ala Gly
    195                      200                      205
Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln Ala Arg
    210                      215                      220

```

Gln Met Val Gln Ala Met Arg Thr Ile Gly Thr His Pro Ser Ser Ser
225 230 235 240
Ala Gly Leu Lys Asp Asp Leu Ile Glu Asn Leu Gln Ala Tyr Gln Lys
245 250 255
Arg Met Gly Val Gln Met Gln Arg Phe Lys
260 265

```
<210> 15
<211> 801
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> PADRE-HBV-s construct encoding fusion of pan DR
      epitope sequence to amino-terminus of hepatitis B
      virus surface antigen gene

<221> CDS
<222> (16)...(765)
<223> PADRE-HBV-s
```

<400> 15	
gctagcgccg ccacc atg gcc aag ttc gtg gct gcc tgg acc ctg aag gct	51
Met Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala	
1 5 10	
gcc gct ctc gag att ggg gga ccc tgc ctg aac gcc gag aac atc aca	99
Ala Ala Leu Glu Ile Gly Gly Pro Cys Leu Asn Ala Glu Asn Ile Thr	
15 20 25	
tca gga ttc cta gga ccc ctt ctc gtg tta cag gcg ggg ttt ttc ttg	147
Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu	
30 35 40	
ttg aca aga atc ctc aca ata ccg cag agt cta gac tcg tgg tgg act	195
Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr	
45 50 55 60	
tct ctc aat ttt cta ggg gga act acc gtg tgt ctt ggc caa aat tcg	243
Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys Leu Gly Gln Asn Ser	
65 70 75	
cag tcc cca acc tcc aat cac tca cca acc tct tgt cct cca act tgt	291
Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys	
80 85 90	
cct ggt tat cgc tgg atg tgt ctg cgg cgt ttt atc atc ttc ctc ttc	339
Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe	
95 100 105	
atc ctg ctg cta tgc ctc atc ttc ttg ttg gtt ctt ctg gac tat caa	387
Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln	
110 115 120	
ggt atg ttg ccc gtt tgt cct cta att cca gga tcc tca aca acc agc	435
Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser	
125 130 135 140	

acg gga cca tgc cgg acc tgc atg act act gct caa gga acc tct atg 483
 Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala Gln Gly Thr Ser Met
 145 150 155

tat ccc tcc tgt tgc tgt acc aaa cct tgc gac gga aat tgc acc tgt 531
 Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys
 160 165 170

att ccc atc cca tca tcc tgg gct ttc gga aaa ttc cta tgg gag tgg 579
 Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp
 175 180 185

gcc tca gcc cgt ttc tcc tgg ctc agt tta cta gtg cca ttt gtt cag 627
 Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu Val Pro Phe Val Gln
 190 195 200

tgg ttc gta ggg ctt tcc ccc act gtt tgg ctt tca gtt ata tgg atg 675
 Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val Ile Trp Met
 205 210 215 220

atg tgg tat tgg ggg cca agt ctg tac agc atc ttg agt ccc ttt tta 723
 Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile Leu Ser Pro Phe Leu
 225 230 235

ccg ctg tta cca att ttc ttt tgt ctt tgg gta tac att taaaccctaa 772
 Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
 240 245

caaaacaaag agatgggggtt actctctaa 801

<210> 16
 <211> 249
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> PADRE-HBV-s

<400> 16
 Met Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Leu Glu
 1 5 10 15
 Ile Gly Gly Pro Cys Leu Asn Ala Glu Asn Ile Thr Ser Gly Phe Leu
 20 25 30
 Gly Pro Leu Leu Val Leu Gln Ala Gly Phe Phe Leu Leu Thr Arg Ile
 35 40 45
 Leu Thr Ile Pro Gln Ser Leu Asp Ser Trp Trp Thr Ser Leu Asn Phe
 50 55 60
 Leu Gly Gly Thr Thr Val Cys Leu Gly Gln Asn Ser Gln Ser Pro Thr
 65 70 75 80
 Ser Asn His Ser Pro Thr Ser Cys Pro Pro Thr Cys Pro Gly Tyr Arg
 85 90 95
 Trp Met Cys Leu Arg Arg Phe Ile Ile Phe Leu Phe Ile Leu Leu Leu
 100 105 110
 Cys Leu Ile Phe Leu Leu Val Leu Leu Asp Tyr Gln Gly Met Leu Pro
 115 120 125
 Val Cys Pro Leu Ile Pro Gly Ser Ser Thr Thr Ser Thr Gly Pro Cys
 130 135 140
 Arg Thr Cys Met Thr Thr Ala Gln Gly Thr Ser Met Tyr Pro Ser Cys
 145 150 155 160

Cys Cys Thr Lys Pro Ser Asp Gly Asn Cys Thr Cys Ile Pro Ile Pro
 165 170 175
 Ser Ser Trp Ala Phe Gly Lys Phe Leu Trp Glu Trp Ala Ser Ala Arg
 180 185 190
 Phe Ser Trp Leu Ser Leu Leu Val Pro Phe Val Gln Trp Phe Val Gly
 195 200 205
 Leu Ser Pro Thr Val Trp Leu Ser Val Ile Trp Met Met Trp Tyr Trp
 210 215 220
 Gly Pro Ser Leu Tyr Ser Ile Leu Ser Pro Phe Leu Pro Leu Leu Pro
 225 230 235 240
 Ile Phe Phe Cys Leu Trp Val Tyr Ile
 245

<210> 17
 <211> 516
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ig-alphaTh construct encoding fusion of the signal
 sequence of Ig-alpha protein fused to multiple MHC
 class II epitopes and the transmembrane and
 cytoplasmic domains of the Ig-alpha protein

<221> CDS
 <222> (16)...(510)
 <223> Ig-alphaTh

<400> 17
 gctagcgccg ccacc atg cca ggg ggt cta gaa gcc ctc aga gcc ctg cct 51
 Met Pro Gly Gly Leu Glu Ala Leu Arg Ala Leu Pro
 1 5 10

 ctc ctc ctc ttc ttg tca tac gcc tgt ttg ggt ccc gga tgc cag gcc 99
 Leu Leu Leu Phe Leu Ser Tyr Ala Cys Leu Gly Pro Gly Cys Gln Ala
 15 20 25

 atc agc cag gct gtg cac gcc gct cac gcc gaa atc aac gaa gct gga 147
 Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
 30 35 40

 aga acc cct cca gct tat cgc cct cca aac gct cct atc ctg ttc ttt 195
 Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe
 45 50 55 60

 ctg ctg acc aga atc ctg aca atc ccc cag tcc ctg gac gcc aag ttc 243
 Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe
 65 70 75

 gtg gct gcc tgg acc ctg aag gct gcc gct ggg atc atc ttg ctg ttc 291
 Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Gly Ile Ile Leu Leu Phe
 80 85 90

 tgt gca gtg gtg cca ggg acg ctg ctg cta ttc agg aaa cgg tgg caa 339
 Cys Ala Val Val Pro Gly Thr Leu Leu Leu Phe Arg Lys Arg Trp Gln
 95 100 105

aat gag aag ttt ggg gtg gac atg cca gat gac tat gaa gat gaa aat 387
 Asn Glu Lys Phe Gly Val Asp Met Pro Asp Asp Tyr Glu Asp Glu Asn
 110 115 120

ctc tat gag ggc ctg aac ctt gat gac tgt tct atg tat gag gac atc 435
 Leu Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile
 125 130 135 140

tcc agg gga ctc cag ggc acc tac cag gat gtg ggc aac ctc cac att 483
 Ser Arg Gly Leu Gln Gly Thr Tyr Gln Asp Val Gly Asn Leu His Ile
 145 150 155

gga gat gcc cag ctg gaa aag cca tgaggtacc 516
 Gly Asp Ala Gln Leu Glu Lys Pro
 160

<210> 18
 <211> 164
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Ig-alphaTh

<400> 18
 Met Pro Gly Gly Leu Glu Ala Leu Arg Ala Leu Pro Leu Leu Leu Phe
 1 5 10 15
 Leu Ser Tyr Ala Cys Leu Gly Pro Gly Cys Gln Ala Ile Ser Gln Ala
 20 25 30
 Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro
 35 40 45
 Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg
 50 55 60
 Ile Leu Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe Val Ala Ala Trp
 65 70 75 80
 Thr Leu Lys Ala Ala Ala Gly Ile Ile Leu Leu Phe Cys Ala Val Val
 85 90 95
 Pro Gly Thr Leu Leu Leu Phe Arg Lys Arg Trp Gln Asn Glu Lys Phe
 100 105 110
 Gly Val Asp Met Pro Asp Asp Tyr Glu Asp Glu Asn Leu Tyr Glu Gly
 115 120 125
 Leu Asn Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile Ser Arg Gly Leu
 130 135 140
 Gln Gly Thr Tyr Gln Asp Val Gly Asn Leu His Ile Gly Asp Ala Gln
 145 150 155 160
 Leu Glu Lys Pro

<210> 19
 <211> 480
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ig-betaTh construct encoding fusion of the signal
 sequence of Ig-beta protein fused to multiple MHC
 class II epitopes and the transmembrane and
 cytoplasmic domains of the Ig-beta protein

<221> CDS
 <222> (16)...(474)
 <223> Ig-betaTh

<400> 19

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gctagcgccg ccacc atg gcc aca ctg gtg ctg tct tcc atg ccc tgc cac      51
      Met Ala Thr Leu Val Leu Ser Ser Met Pro Cys His
              1              5              10

tgg ctg ttg ttc ctg ctg ctg ctc ttc tca ggt gag ccg atc agc cag      99
Trp Leu Leu Phe Leu Leu Leu Phe Ser Gly Glu Pro Ile Ser Gln
      15              20              25

gct gtg cac gcc gct cac gcc gaa atc aac gaa gct gga aga acc cct      147
Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly Arg Thr Pro
      30              35              40

cca gct tat cgc cct cca aac gct cct atc ctg ttc ttt ctg ctg acc      195
Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe Leu Leu Thr
      45              50              55              60

aga atc ctg aca atc ccc cag tcc ctg gac gcc aag ttc gtg gct gcc      243
Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe Val Ala Ala
              65              70              75

tgg acc ctg aag gct gcc gct att atc ttg atc cag acc ctc ctc atc      291
Trp Thr Leu Lys Ala Ala Ala Ile Ile Leu Ile Gln Thr Leu Leu Ile
              80              85              90

atc ctc ttc atc att gtg ccc atc ttc ctg cta ctt gac aag gat gac      339
Ile Leu Phe Ile Ile Val Pro Ile Phe Leu Leu Leu Asp Lys Asp Asp
              95              100              105

ggc aag gct ggg atg gag gaa gat cac acc tat gag ggc ttg aac att      387
Gly Lys Ala Gly Met Glu Glu Asp His Thr Tyr Glu Gly Leu Asn Ile
      110              115              120

gac cag aca gcc acc tat gaa gac ata gtg act ctt cgg aca ggg gag      435
Asp Gln Thr Ala Thr Tyr Glu Asp Ile Val Thr Leu Arg Thr Gly Glu
      125              130              135              140

gta aag tgg tcg gta gga gag cat cca ggc cag gaa tgaggtacc      480
Val Lys Trp Ser Val Gly Glu His Pro Gly Gln Glu
              145              150

```

<210> 20
 <211> 152
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Ig-betaTh

<400> 20

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Met Ala Thr Leu Val Leu Ser Ser Met Pro Cys His Trp Leu Leu Phe
      1              5              10              15

```

```

Leu Leu Leu Leu Phe Ser Gly Glu Pro Ile Ser Gln Ala Val His Ala
      20                      25                      30
Ala His Ala Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg
      35                      40                      45
Pro Pro Asn Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr
      50                      55                      60
Ile Pro Gln Ser Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys
65                      70                      75                      80
Ala Ala Ala Ile Ile Leu Ile Gln Thr Leu Leu Ile Ile Leu Phe Ile
      85                      90                      95
Ile Val Pro Ile Phe Leu Leu Leu Asp Lys Asp Asp Gly Lys Ala Gly
      100                     105                     110
Met Glu Glu Asp His Thr Tyr Glu Gly Leu Asn Ile Asp Gln Thr Ala
      115                     120                     125
Thr Tyr Glu Asp Ile Val Thr Leu Arg Thr Gly Glu Val Lys Trp Ser
      130                     135                     140
Val Gly Glu His Pro Gly Gln Glu
145                      150

```

```

<210> 21
<211> 264
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> SigTh construct encoding fusion of the signal
sequence of kappa immunoglobulin fused to multiple
MHC class II epitopes

```

```

<221> CDS
<222> (16)...(258)
<223> SigTh

```

```

<400> 21
gctagcgccg ccacc atg gga atg cag gtg cag atc cag agc ctg ttt ctg      51
      Met Gly Met Gln Val Gln Ile Gln Ser Leu Phe Leu
      1                      5                      10

ctc ctc ctg tgg gtg ccc ggg tcc cga gga atc agc cag gct gtg cac      99
Leu Leu Leu Trp Val Pro Gly Ser Arg Gly Ile Ser Gln Ala Val His
      15                      20                      25

gcc gct cac gcc gaa atc aac gaa gct gga aga acc cct cca gct tat      147
Ala Ala His Ala Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr
      30                      35                      40

cgc cct cca aac gct cct atc ctg ttc ttt ctg ctg acc aga atc ctg      195
Arg Pro Pro Asn Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu
      45                      50                      55                      60

aca atc ccc cag tcc ctg gac gcc aag ttc gtg gct gcc tgg acc ctg      243
Thr Ile Pro Gln Ser Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu
      65                      70                      75

aag gct gcc gct tgaggtacc      264
Lys Ala Ala Ala
      80

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<210> 22
 <211> 80
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> SigTh

<400> 22
 Met Gly Met Gln Val Gln Ile Gln Ser Leu Phe Leu Leu Leu Trp
 1 5 10 15
 Val Pro Gly Ser Arg Gly Ile Ser Gln Ala Val His Ala Ala His Ala
 20 25 30
 Glu Ile Asn Glu Ala Gly Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn
 35 40 45
 Ala Pro Ile Leu Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln
 50 55 60
 Ser Leu Asp Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
 65 70 75 80

<210> 23
 <211> 723
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (8)...(706)
 <223> human HLA-DR invariant chain (Ii) protein

<400> 23
 ttcccag atg cac agg agg aga agc agg agc tgt cgg gaa gat cag aag 49
 Met His Arg Arg Arg Ser Arg Ser Cys Arg Glu Asp Gln Lys
 1 5 10
 cca gtc atg gat gac cag cgc gac ctt atc tcc aac aat gag caa ctg 97
 Pro Val Met Asp Asp Gln Arg Asp Leu Ile Ser Asn Asn Glu Gln Leu
 15 20 25 30
 ccc atg ctg ggc cgg cgc cct ggg gcc ccg gag agc aag tgc agc cgc 145
 Pro Met Leu Gly Arg Arg Pro Gly Ala Pro Glu Ser Lys Cys Ser Arg
 35 40 45
 gga gcc ctg tac aca ggc ttt tcc atc ctg gtg act ctg ctc ctc gct 193
 Gly Ala Leu Tyr Thr Gly Phe Ser Ile Leu Val Thr Leu Leu Leu Ala
 50 55 60
 ggc cag gcc acc acc gcc tac ttc ctg tac cag cag cag ggc cgg ctg 241
 Gly Gln Ala Thr Thr Ala Tyr Phe Leu Tyr Gln Gln Gln Gly Arg Leu
 65 70 75
 gac aaa ctg aca gtc acc tcc cag aac ctg cag ctg gag aac ctg cgc 289
 Asp Lys Leu Thr Val Thr Ser Gln Asn Leu Gln Leu Glu Asn Leu Arg
 80 85 90
 atg aag ctt ccc aag cct ccc aag cct gtg agc aag atg cgc atg gcc 337
 Met Lys Leu Pro Lys Pro Pro Lys Pro Val Ser Lys Met Arg Met Ala
 95 100 105 110

```

acc ccg ctg ctg atg cag gcg ctg ccc atg gga gcc ctg ccc cag ggg      385
Thr Pro Leu Leu Met Gln Ala Leu Pro Met Gly Ala Leu Pro Gln Gly
          115                120                125

ccc atg cag aat gcc acc aag tat ggc aac atg aca gag gac cat gtg      433
Pro Met Gln Asn Ala Thr Lys Tyr Gly Asn Met Thr Glu Asp His Val
          130                135                140

atg cac ctg ctc cag aat gct gac ccc ctg aag gtg tac ccg cca ctg      481
Met His Leu Leu Gln Asn Ala Asp Pro Leu Lys Val Tyr Pro Pro Leu
          145                150                155

aag ggg agc ttc ccg gag aac ctg aga cac ctt aag aac acc atg gag      529
Lys Gly Ser Phe Pro Glu Asn Leu Arg His Leu Lys Asn Thr Met Glu
          160                165                170

acc ata gac tgg aag gtc ttt gag agc tgg atg cac cat tgg ctc ctg      577
Thr Ile Asp Trp Lys Val Phe Glu Ser Trp Met His His Trp Leu Leu
          175                180                185                190

ttt gaa atg agc agg cac tcc ttg gag caa aag ccc act gac gct cca      625
Phe Glu Met Ser Arg His Ser Leu Glu Gln Lys Pro Thr Asp Ala Pro
          195                200                205

ccg aaa gag tca ctg gaa ctg gag gac ccg tct tct ggg ctg ggt gtg      673
Pro Lys Glu Ser Leu Glu Leu Glu Asp Pro Ser Ser Gly Leu Gly Val
          210                215                220

acc aag cag gat ctg ggc cca gtc ccc atg tgagagcagc agaggcggtc      723
Thr Lys Gln Asp Leu Gly Pro Val Pro Met
          225                230

```

<210> 24
 <211> 232
 <212> PRT
 <213> Homo sapiens

<220>
 <223> human HLA-DR invariant chain (Ii) protein

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<400> 24
Met His Arg Arg Arg Ser Arg Ser Cys Arg Glu Asp Gln Lys Pro Val
 1          5          10          15
Met Asp Asp Gln Arg Asp Leu Ile Ser Asn Asn Glu Gln Leu Pro Met
          20          25          30
Leu Gly Arg Arg Pro Gly Ala Pro Glu Ser Lys Cys Ser Arg Gly Ala
          35          40          45
Leu Tyr Thr Gly Phe Ser Ile Leu Val Thr Leu Leu Leu Ala Gly Gln
          50          55          60
Ala Thr Thr Ala Tyr Phe Leu Tyr Gln Gln Gln Gly Arg Leu Asp Lys
          65          70          75          80
Leu Thr Val Thr Ser Gln Asn Leu Gln Leu Glu Asn Leu Arg Met Lys
          85          90          95
Leu Pro Lys Pro Pro Lys Pro Val Ser Lys Met Arg Met Ala Thr Pro
          100         105         110
Leu Leu Met Gln Ala Leu Pro Met Gly Ala Leu Pro Gln Gly Pro Met
          115         120         125
Gln Asn Ala Thr Lys Tyr Gly Asn Met Thr Glu Asp His Val Met His
          130         135         140

```

Leu Leu Gln Asn Ala Asp Pro Leu Lys Val Tyr Pro Pro Leu Lys Gly
 145 150 155 160
 Ser Phe Pro Glu Asn Leu Arg His Leu Lys Asn Thr Met Glu Thr Ile
 165 170 175
 Asp Trp Lys Val Phe Glu Ser Trp Met His His Trp Leu Leu Phe Glu
 180 185 190
 Met Ser Arg His Ser Leu Glu Gln Lys Pro Thr Asp Ala Pro Pro Lys
 195 200 205
 Glu Ser Leu Glu Leu Glu Asp Pro Ser Ser Gly Leu Gly Val Thr Lys
 210 215 220
 Gln Asp Leu Gly Pro Val Pro Met
 225 230

<210> 25
 <211> 1298
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (11)...(1261)
 <223> human lysosomal membrane glycoprotein-1 (LAMP-1)

<400> 25
 ccgcctcggc atg gcg ccc cgc agc gcc cgg cga ccc ctg ctg ctg cta 49
 Met Ala Pro Arg Ser Ala Arg Arg Pro Leu Leu Leu Leu
 1 5 10

 ctg cct gtt gct gct gct cgg cct cat gca ttg tcg tca gca gcc atg 97
 Leu Pro Val Ala Ala Ala Arg Pro His Ala Leu Ser Ser Ala Ala Met
 15 20 25

 ttt atg gtg aaa aat ggc aac ggg acc gcg tgc ata atg gcc aac ttc 145
 Phe Met Val Lys Asn Gly Asn Gly Thr Ala Cys Ile Met Ala Asn Phe
 30 35 40 45

 tct gct gcc ttc tca gtg aac tac gac acc aag agt ggc ccc aag aac 193
 Ser Ala Ala Phe Ser Val Asn Tyr Asp Thr Lys Ser Gly Pro Lys Asn
 50 55 60

 atg acc ttt gac ctg cca tca gat gcc aca gtg gtg ctc aac cgc agc 241
 Met Thr Phe Asp Leu Pro Ser Asp Ala Thr Val Val Leu Asn Arg Ser
 65 70 75

 tcc tgt gga aaa gag aac act tct gac ccc agt ctc gtg att gct ttt 289
 Ser Cys Gly Lys Glu Asn Thr Ser Asp Pro Ser Leu Val Ile Ala Phe
 80 85 90

 gga aga gga cat aca ctc act ctc aat ttc acg aga aat gca aca cgt 337
 Gly Arg Gly His Thr Leu Thr Leu Asn Phe Thr Arg Asn Ala Thr Arg
 95 100 105

 tac agc gtt cag ctc atg agt ttt gtt tat aac ttg tca gac aca cac 385
 Tyr Ser Val Gln Leu Met Ser Phe Val Tyr Asn Leu Ser Asp Thr His
 110 115 120 125

ctt ttc ccc aat gcg agc tcc aaa gaa atc aag act gtg gaa tct ata Leu Phe Pro Asn Ala Ser Ser Lys Glu Ile Lys Thr Val Glu Ser Ile	433
130 135 140	
act gac atc agg gca gat ata gat aaa aaa tac aga tgt gtt agt ggc Thr Asp Ile Arg Ala Asp Ile Asp Lys Lys Tyr Arg Cys Val Ser Gly	481
145 150 155	
acc cag gtc cac atg aac aac gtg acc gta acg ctc cat gat gcc acc Thr Gln Val His Met Asn Asn Val Thr Val Thr Leu His Asp Ala Thr	529
160 165 170	
atc cag gcg tac ctt tcc aac agc agc ttc agc agg gga gag aca cgc Ile Gln Ala Tyr Leu Ser Asn Ser Ser Phe Ser Arg Gly Glu Thr Arg	577
175 180 185	
tgt gaa caa gac agg cct tcc cca acc aca gcg ccc cct gcg cca ccc Cys Glu Gln Asp Arg Pro Ser Pro Thr Thr Ala Pro Pro Ala Pro Pro	625
190 195 200 205	
agc ccc tcg ccc tca ccc gtg ccc aag agc ccc tct gtg gac aag tac Ser Pro Ser Pro Ser Pro Val Pro Lys Ser Pro Ser Val Asp Lys Tyr	673
210 215 220	
aac gtg agc ggc acc aac ggg acc tgc ctg ctg gcc agc atg ggg ctg Asn Val Ser Gly Thr Asn Gly Thr Cys Leu Leu Ala Ser Met Gly Leu	721
225 230 235	
cag ctg aac ctc acc tat gag agg aag gac aac acg acg gtg aca agg Gln Leu Asn Leu Thr Tyr Glu Arg Lys Asp Asn Thr Thr Val Thr Arg	769
240 245 250	
ctt ctc aac atc aac ccc aac aag acc tcg gcc agc ggg agc tgc ggc Leu Leu Asn Ile Asn Pro Asn Lys Thr Ser Ala Ser Gly Ser Cys Gly	817
255 260 265	
gcc cac ctg gtg act ctg gag ctg cac agc gag ggc acc acc gtc ctg Ala His Leu Val Thr Leu Glu Leu His Ser Glu Gly Thr Thr Val Leu	865
270 275 280 285	
ctc ttc cag ttc ggg atg aat gca agt tct agc cgg ttt ttc cta caa Leu Phe Gln Phe Gly Met Asn Ala Ser Ser Ser Arg Phe Phe Leu Gln	913
290 295 300	
gga atc cag ttg aat aca att ctt cct gac gcc aga gac cct gcc ttt Gly Ile Gln Leu Asn Thr Ile Leu Pro Asp Ala Arg Asp Pro Ala Phe	961
305 310 315	
aaa gct gcc aac ggc tcc ctg cga gcg ctg cag gcc aca gtc ggc aat Lys Ala Ala Asn Gly Ser Leu Arg Ala Leu Gln Ala Thr Val Gly Asn	1009
320 325 330	
tcc tac aag tgc aac gcg gag gag cac gtc cgt gtc acg aag gcg ttt Ser Tyr Lys Cys Asn Ala Glu Glu His Val Arg Val Thr Lys Ala Phe	1057
335 340 345	

tca gtc aat ata ttc aaa gtg tgg gtc cag gct ttc aag gtg gaa ggt 1105
 Ser Val Asn Ile Phe Lys Val Trp Val Gln Ala Phe Lys Val Glu Gly
 350 355 360 365

ggc cag ttt ggc tct gtg gag gag tgt ctg ctg gac gag aac agc acg 1153
 Gly Gln Phe Gly Ser Val Glu Glu Cys Leu Leu Asp Glu Asn Ser Thr
 370 375 380

ctg atc ccc atc gct gtg ggt ggt gcc ctg gcg ggg ctg gtc ctc atc 1201
 Leu Ile Pro Ile Ala Val Gly Gly Ala Leu Ala Gly Leu Val Leu Ile
 385 390 395

gtc ctc atc gcc tac ctc gtc ggc agg aag agg agt cac gca ggc tac 1249
 Val Leu Ile Ala Tyr Leu Val Gly Arg Lys Arg Ser His Ala Gly Tyr
 400 405 410

cag act atc tagcctgggtg cagcagaggca cagcagctgc aggggcctct 1298
 Gln Thr Ile
 415

<210> 26
 <211> 416
 <212> PRT
 <213> Homo sapiens

<220>
 <223> human lysosomal membrane glycoprotein-1 (LAMP-1)

<400> 26
 Met Ala Pro Arg Ser Ala Arg Arg Pro Leu Leu Leu Leu Leu Pro Val
 1 5 10 15
 Ala Ala Ala Arg Pro His Ala Leu Ser Ser Ala Ala Met Phe Met Val
 20 25 30
 Lys Asn Gly Asn Gly Thr Ala Cys Ile Met Ala Asn Phe Ser Ala Ala
 35 40 45

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

Gly Thr Asn Gly Thr Cys Leu Leu Ala Ser Met Gly Leu Gln Leu Asn
 225 230 235 240
 Leu Thr Tyr Glu Arg Lys Asp Asn Thr Thr Val Thr Arg Leu Leu Asn
 245 250 255
 Ile Asn Pro Asn Lys Thr Ser Ala Ser Gly Ser Cys Gly Ala His Leu
 260 265 270
 Val Thr Leu Glu Leu His Ser Glu Gly Thr Thr Val Leu Leu Phe Gln
 275 280 285
 Phe Gly Met Asn Ala Ser Ser Ser Arg Phe Phe Leu Gln Gly Ile Gln
 290 295 300
 Leu Asn Thr Ile Leu Pro Asp Ala Arg Asp Pro Ala Phe Lys Ala Ala
 305 310 315 320
 Asn Gly Ser Leu Arg Ala Leu Gln Ala Thr Val Gly Asn Ser Tyr Lys
 325 330 335
 Cys Asn Ala Glu Glu His Val Arg Val Thr Lys Ala Phe Ser Val Asn
 340 345 350
 Ile Phe Lys Val Trp Val Gln Ala Phe Lys Val Glu Gly Gly Gln Phe
 355 360 365
 Gly Ser Val Glu Glu Cys Leu Leu Asp Glu Asn Ser Thr Leu Ile Pro
 370 375 380
 Ile Ala Val Gly Gly Ala Leu Ala Gly Leu Val Leu Ile Val Leu Ile
 385 390 395 400
 Ala Tyr Leu Val Gly Arg Lys Arg Ser His Ala Gly Tyr Gln Thr Ile
 405 410 415

<210> 27
 <211> 792
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)...(792)
 <223> human HLA-DMB

<400> 27
 atg atc aca ttc ctg ccg ctg ctg ctg ggg ctc agc ctg ggc tgc aca 48
 Met Ile Thr Phe Leu Pro Leu Leu Leu Gly Leu Ser Leu Gly Cys Thr
 1 5 10 15
 gga gca ggt ggc ttc gtg gcc cat gtg gaa agc acc tgt ctg ttg gat 96
 Gly Ala Gly Gly Phe Val Ala His Val Glu Ser Thr Cys Leu Leu Asp
 20 25 30
 gat gct ggg act cca aag gat ttc aca tac tgc atc tcc ttc aac aag 144
 Asp Ala Gly Thr Pro Lys Asp Phe Thr Tyr Cys Ile Ser Phe Asn Lys
 35 40 45
 gat ctg ctg acc tgc tgg gat cca gag gag aat aag atg gcc cct tgc 192
 Asp Leu Leu Thr Cys Trp Asp Pro Glu Glu Asn Lys Met Ala Pro Cys
 50 55 60
 gaa ttt ggg gtg ctg aat agc ttg gcg aat gtc ctc tca cag cac ctc 240
 Glu Phe Gly Val Leu Asn Ser Leu Ala Asn Val Leu Ser Gln His Leu
 65 70 75 80
 aac caa aaa gac acc ctg atg cag cgc ttg cgc aat ggg ctt cag aat 288
 Asn Gln Lys Asp Thr Leu Met Gln Arg Leu Arg Asn Gly Leu Gln Asn
 85 90 95

tgt gcc aca cac acc cag ccc ttc tgg gga tca ctg acc aac agg aca 336
 Cys Ala Thr His Thr Gln Pro Phe Trp Gly Ser Leu Thr Asn Arg Thr
 100 105 110

cgg cca cca tct gtg caa gta gcc aaa acc act cct ttt aac acg agg 384
 Arg Pro Pro Ser Val Gln Val Ala Lys Thr Thr Pro Phe Asn Thr Arg
 115 120 125

gag cct gtg atg ctg gcc tgc tat gtg tgg ggc ttc tat cca gca gaa 432
 Glu Pro Val Met Leu Ala Cys Tyr Val Trp Gly Phe Tyr Pro Ala Glu
 130 135 140

gtg act atc acg tgg agg aag aac ggg aag ctt gtc atg cct cac agc 480
 Val Thr Ile Thr Trp Arg Lys Asn Gly Lys Leu Val Met Pro His Ser
 145 150 155 160

agt gcg cac aag act gcc cag ccc aat gga gac tgg aca tac cag acc 528
 Ser Ala His Lys Thr Ala Gln Pro Asn Gly Asp Trp Thr Tyr Gln Thr
 165 170 175

ctc tcc cat tta gcc tta acc ccc tct tac ggg gac act tac acc tgt 576
 Leu Ser His Leu Ala Leu Thr Pro Ser Tyr Gly Asp Thr Tyr Thr Cys
 180 185 190

gtg gta gag cac att ggg gct cct gag ccc atc ctt cgg gac tgg aca 624
 Val Val Glu His Ile Gly Ala Pro Glu Pro Ile Leu Arg Asp Trp Thr
 195 200 205

cct ggg ctg tcc ccc atg cag acc ctg aag gtt tct gtg tct gca gtg 672
 Pro Gly Leu Ser Pro Met Gln Thr Leu Lys Val Ser Val Ser Ala Val
 210 215 220

act ctg ggc ctg ggc ctc atc atc ttc tct ctt ggt gtg atc agc tgg 720
 Thr Leu Gly Leu Gly Leu Ile Ile Phe Ser Leu Gly Val Ile Ser Trp
 225 230 235 240

cgg aga gct ggc cac tct agt tac act cct ctt cct ggg tcc aat tat 768
 Arg Arg Ala Gly His Ser Ser Tyr Thr Pro Leu Pro Gly Ser Asn Tyr
 245 250 255

tca gaa gga tgg cac att tcc tag 792
 Ser Glu Gly Trp His Ile Ser
 260

<210> 28
 <211> 263
 <212> PRT
 <213> Homo sapiens

<220>
 <223> human HLA-DMB

<400> 28

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 Gly Ala Gly Gly Phe Val Ala His Val Glu Ser Thr Cys Leu Asp
 20 25 30

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

<210> 29
 <211> 822
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)...(822)
 <223> human HLA-DO beta

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 Met Gly Ser Gly Trp Val Pro Trp Val Val Ala Leu Leu Val Asn Leu
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 acc caa ctg gat tcc tcc atg act caa ggc aca gac tct cca gaa gat 96
 Thr Gln Leu Asp Ser Ser Met Thr Gln Gly Thr Asp Ser Pro Glu Asp
 20 25 30
 ttt gtg att cag gca aag gct gac tgt tac ttc acc aac ggg aca gaa 144
 Phe Val Ile Gln Ala Lys Ala Asp Cys Tyr Phe Thr Asn Gly Thr Glu
 35 40 45
 aag gtg cag ttt gtg gtc aga ttc atc ttt aac ttg gag gag tat gta 192
 Lys Val Gln Phe Val Val Arg Phe Ile Phe Asn Leu Glu Glu Tyr Val
 50 55 60

cgt ttc gac agt gat gtg ggg atg ttt gtg gca ttg acc aag ctg ggg Arg Phe Asp Ser Asp Val Gly Met Phe Val Ala Leu Thr Lys Leu Gly 65 70 75 80	240
cag cca gat gct gag cag tgg aac agc cgg ctg gat ctc ttg gag agg Gln Pro Asp Ala Glu Gln Trp Asn Ser Arg Leu Asp Leu Leu Glu Arg 85 90 95	288
agc aga cag gcc gtg gat ggg gtc tgt aga cac aac tac agg ctg ggc Ser Arg Gln Ala Val Asp Gly Val Cys Arg His Asn Tyr Arg Leu Gly 100 105 110	336
gca ccc ttc act gtg ggg aga aaa gtg caa cca gag gtg aca gtg tac Ala Pro Phe Thr Val Gly Arg Lys Val Gln Pro Glu Val Thr Val Tyr 115 120 125	384
cca gag agg acc cca ctc ctg cac cag cat aat ctg ctg cac tgc tct Pro Glu Arg Thr Pro Leu Leu His Gln His Asn Leu Leu His Cys Ser 130 135 140	432
gtg aca ggc ttc tat cca ggg gat atc aag atc aag tgg ttc ctg aat Val Thr Gly Phe Tyr Pro Gly Asp Ile Lys Ile Lys Trp Phe Leu Asn 145 150 155 160	480
ggg cag gag gag aga gct ggg gtc atg tcc act ggc cct atc agg aat Gly Gln Glu Glu Arg Ala Gly Val Met Ser Thr Gly Pro Ile Arg Asn 165 170 175	528
gga gac tgg acc ttt cag act gtg gtg atg cta gaa atg act cct gaa Gly Asp Trp Thr Phe Gln Thr Val Val Met Leu Glu Met Thr Pro Glu 180 185 190	576
ctt gga cat gtc tac acc tgc ctt gtc gat cac tcc agc ctg ctg agc Leu Gly His Val Tyr Thr Cys Leu Val Asp His Ser Ser Leu Leu Ser 195 200 205	624
cct gtt tct gtg gag tgg aga gct cag tct gaa tat tct tgg aga aag Pro Val Ser Val Glu Trp Arg Ala Gln Ser Glu Tyr Ser Trp Arg Lys 210 215 220	672
atg ctg agt ggc att gca gcc ttc cta ctt ggg cta atc ttc ctt ctg Met Leu Ser Gly Ile Ala Ala Phe Leu Leu Gly Leu Ile Phe Leu Leu 225 230 235 240	720
gtg gga atc gtc atc cag cta agg gct cag aaa gga tat gtg agg acg Val Gly Ile Val Ile Gln Leu Arg Ala Gln Lys Gly Tyr Val Arg Thr 245 250 255	768
cag atg tct ggt aat gag gtc tca aga gct gtt ctg ctc cct cag tca Gln Met Ser Gly Asn Glu Val Ser Arg Ala Val Leu Leu Pro Gln Ser 260 265 270	816
tgc taa Cys	822

<210> 30

<211> 273

<212> PRT

79aa

<213> Homo sapiens

<220>

<223> human HLA-DO beta

<400> 30

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

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<210> 31

<211> 700

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (1)...(681)

<223> human MB-1 Ig-alpha

<400> 31

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

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48

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ctc ctc ttc ctg ctg tct gct gtc tac ctg ggc cct ggg tgc cag gcc      96
Leu Leu Phe Leu Leu Ser Ala Val Tyr Leu Gly Pro Gly Cys Gln Ala
      20                      25                      30

ctg tgg atg cac aag gtc cca gca tca ttg atg gtg agc ctg ggg gaa      144
Leu Trp Met His Lys Val Pro Ala Ser Leu Met Val Ser Leu Gly Glu
      35                      40                      45

gac gcc cac ttc caa tgc ccg cac aat agc agc aac aac gcc aac gtc      192
Asp Ala His Phe Gln Cys Pro His Asn Ser Ser Asn Asn Ala Asn Val
      50                      55                      60

acc tgg tgg cgc gtc ctc cat ggc aac tac acg tgg ccc cct gag ttc      240
Thr Trp Trp Arg Val Leu His Gly Asn Tyr Thr Trp Pro Pro Glu Phe
      65                      70                      75                      80

ttg ggc ccg ggc gag gac ccc aat ggt acg ctg atc atc cag aat gtg      288
Leu Gly Pro Gly Glu Asp Pro Asn Gly Thr Leu Ile Ile Gln Asn Val
      85                      90                      95

aac aag agc cat ggg ggc ata tac gtg tgc cgg gtc cag gag ggc aac      336
Asn Lys Ser His Gly Gly Ile Tyr Val Cys Arg Val Gln Glu Gly Asn
      100                     105                     110

gag tca tac cag cag tcc tgc ggc acc tac ctc cgc gtg cgc cag ccg      384
Glu Ser Tyr Gln Gln Ser Cys Gly Thr Tyr Leu Arg Val Arg Gln Pro
      115                     120                     125

ccc ccc agg ccc ttc ctg gac atg ggg gag ggc acc aag aac cga atc      432
Pro Pro Arg Pro Phe Leu Asp Met Gly Glu Gly Thr Lys Asn Arg Ile
      130                     135                     140

atc aca gcc gag ggg atc atc ctc ctg ttc tgc gcg gtg gtg cct ggg      480
Ile Thr Ala Glu Gly Ile Ile Leu Leu Phe Cys Ala Val Val Pro Gly
      145                     150                     155                     160

acg ctg ctg ctg ttc agg aaa cga tgg cag aac gag aag ctc ggg ttg      528
Thr Leu Leu Leu Phe Arg Lys Arg Trp Gln Asn Glu Lys Leu Gly Leu
      165                     170                     175

gat gcc ggg gat gaa tat gaa gat gaa aac ctt tat gaa ggc ctg aac      576
Asp Ala Gly Asp Glu Tyr Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn
      180                     185                     190

ctg gac gac tgc tcc atg tat gag gac atc tcc cgg ggc ctc cag ggc      624
Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile Ser Arg Gly Leu Gln Gly
      195                     200                     205

acc tac cag gat gtg ggc agc ctc aac ata gga gat gtc cag ctg gag      672
Thr Tyr Gln Asp Val Gly Ser Leu Asn Ile Gly Asp Val Gln Leu Glu
      210                     215                     220

aag ccg tgacacccct actcctgcca gg      700
Lys Pro
225

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<210> 32

<211> 226

79cc

<212> PRT
 <213> Homo sapiens

<220>
 <223> human MB-1 Ig-alpha

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 35 40 45
 Asp Ala His Phe Gln Cys Pro His Asn Ser Ser Asn Asn Ala Asn Val
 50 55 60
 Thr Trp Trp Arg Val Leu His Gly Asn Tyr Thr Trp Pro Pro Glu Phe
 65 70 75 80
 Leu Gly Pro Gly Glu Asp Pro Asn Gly Thr Leu Ile Ile Gln Asn Val
 85 90 95
 Asn Lys Ser His Gly Gly Ile Tyr Val Cys Arg Val Gln Glu Gly Asn
 100 105 110
 Glu Ser Tyr Gln Gln Ser Cys Gly Thr Tyr Leu Arg Val Arg Gln Pro
 115 120 125
 Pro Pro Arg Pro Phe Leu Asp Met Gly Glu Gly Thr Lys Asn Arg Ile
 130 135 140
 Ile Thr Ala Glu Gly Ile Ile Leu Leu Phe Cys Ala Val Val Pro Gly
 145 150 155 160
 Thr Leu Leu Leu Phe Arg Lys Arg Trp Gln Asn Glu Lys Leu Gly Leu
 165 170 175
 Asp Ala Gly Asp Glu Tyr Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn
 180 185 190
 Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile Ser Arg Gly Leu Gln Gly
 195 200 205
 Thr Tyr Gln Asp Val Gly Ser Leu Asn Ile Gly Asp Val Gln Leu Glu
 210 215 220
 Lys Pro
 225

<210> 33
 <211> 723
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (17)...(706)
 <223> human Ig-beta protein

<400> 33
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 Trp Met Val Ala Leu Leu Leu Leu Leu Ser Ala Glu Pro Val Pro Ala
 15 20 25

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gcc aga tcg gag gac cgg tac cgg aat ccc aaa ggt agt gct tgt tcg      148
Ala Arg Ser Glu Asp Arg Tyr Arg Asn Pro Lys Gly Ser Ala Cys Ser
      30                      35                      40

cgg atc tgg cag agc cca cgt ttc ata gcc agg aaa cgg cgc ttc acg      196
Arg Ile Trp Gln Ser Pro Arg Phe Ile Ala Arg Lys Arg Arg Phe Thr
      45                      50                      55                      60

gtg aaa atg cac tgc tac atg aac agc gcc tcc ggc aat gtg agc tgg      244
Val Lys Met His Cys Tyr Met Asn Ser Ala Ser Gly Asn Val Ser Trp
                        65                      70                      75

ctc tgg aag cag gag atg gac gag aat ccc cag cag ctg aag ctg gaa      292
Leu Trp Lys Gln Glu Met Asp Glu Asn Pro Gln Gln Leu Lys Leu Glu
                        80                      85                      90

aag ggc cgc atg gaa gag tcc cag aac gaa tct ctc gcc acc ctc acc      340
Lys Gly Arg Met Glu Glu Ser Gln Asn Glu Ser Leu Ala Thr Leu Thr
                        95                      100                      105

atc caa ggc atc cgg ttt gag gac aat ggc atc tac ttc tgc cag cag      388
Ile Gln Gly Ile Arg Phe Glu Asp Asn Gly Ile Tyr Phe Cys Gln Gln
      110                      115                      120

aag tgc aac aac acc tcg gag gtc tac cag ggc tgc ggc aca gag ctg      436
Lys Cys Asn Asn Thr Ser Glu Val Tyr Gln Gly Cys Gly Thr Glu Leu
      125                      130                      135                      140

cga gtc atg gga ttc agc acc ttg gca cag ctg aag cag agg aac acg      484
Arg Val Met Gly Phe Ser Thr Leu Ala Gln Leu Lys Gln Arg Asn Thr
                        145                      150                      155

ctg aag gat ggt atc atc atg atc cag acg ctg ctg atc atc ctc ttc      532
Leu Lys Asp Gly Ile Ile Met Ile Gln Thr Leu Leu Ile Ile Leu Phe
                        160                      165                      170

atc atc gtg cct atc ttc ctg ctg ctg gac aag gat gac agc aag gct      580
Ile Ile Val Pro Ile Phe Leu Leu Leu Asp Lys Asp Asp Ser Lys Ala
                        175                      180                      185

ggc atg gag gaa gat cac acc tac gag ggc ctg gac att gac cag aca      628
Gly Met Glu Glu Asp His Thr Tyr Glu Gly Leu Asp Ile Asp Gln Thr
      190                      195                      200

gcc acc tat gag gac ata gtg acg ctg cgg aca ggg gaa gtg aag tgg      676
Ala Thr Tyr Glu Asp Ile Val Thr Leu Arg Thr Gly Glu Val Lys Trp
      205                      210                      215                      220

tct gta ggt gag cac cca ggc cag gag tgagagccag gtcgccccat      723
Ser Val Gly Glu His Pro Gly Gln Glu
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<210> 34
<211> 229
<212> PRT
<213> Homo sapiens

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<220>
<223> human Ig-beta protein

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<400> 34

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

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<210> 35

<211> 5053

<212> DNA

<213> Artificial Sequence

<220>

<223> vector pEP2

<400> 35

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cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc      180
ttaggggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt      240
gattattgac tagttattaa tagtaatcaa ttacgggggtc attagttcat agcccatata      300
tggagttccg cgttacataa cttacggtaa atggcccgcg tggctgaccg cccaacgacc      360
cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc      420
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ctcgaggccg	cgattaaatt	ccaacatgga	tgctgattta	tatgggtata	aatgggctcg	4200
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agaagaatat	cctgattcag	gtgaaaatat	tgttgatgcg	ctggcagtg	tcctgcgccg	4500

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gttgcatteg attcctgttt gtaattgtcc ttttaacagc gatcgcggtat ttcgtctcgc 4560
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taatggctgg cctgttgaac aagtctggaa agaaatgcat aaacttttgc cattctcacc 4680
ggattcagtc gtcactcatg gtgatttctc acttgataac cttatttttg acgaggggaa 4740
attaataggt tgtattgatg ttggacgagt cggaatcgca gaccgatacc aggatcttgc 4800
catcctatgg aactgcctcg gtgagttttc tccttcatta cagaaacggc tttttcaaaa 4860
atatggtatt gataatcctg atatgaataa attgcagttt catttgatgc togatgagtt 4920
tttctaatac gaattgggta attggttgta acactggcag agcatcatga gcggatacat 4980
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<210> 36
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<212> DNA
<213> Artificial Sequence

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<220>
<223> vector pMIN.0

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<221> CDS
<222> (16)...(411)

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<400> 36

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1 5 10

ctc ctc ctg tgg gtg ccc ggg tcc aga gga cac acc ctg tgg aag gcc 99
Leu Leu Leu Trp Val Pro Gly Ser Arg Gly His Thr Leu Trp Lys Ala
15 20 25

gga atc ctg tat aag gcc aag ttc gtg gct gcc tgg acc ctg aag gct 147
Gly Ile Leu Tyr Lys Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala
30 35 40

gcc gct ttc ctg cct agc gat ttc ttt cct agc gtg aag ctg acc cca 195
Ala Ala Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Lys Leu Thr Pro
45 50 55 60

ctg tgc gtg acc ctg tat atg gat gac gtg gtg ctg gga gcc agc atc 243
Leu Cys Val Thr Leu Tyr Met Asp Asp Val Val Leu Gly Ala Ser Ile
65 70 75

atc aac ttc gag aag ctg gga ctg tcc aga tac gtg gct agg ctg atc 291
Ile Asn Phe Glu Lys Leu Gly Leu Ser Arg Tyr Val Ala Arg Leu Ile
80 85 90

ctg aag gag cct gtg cac ggc gtg tcc acc ctg cca gag acc acc gtg 339
Leu Lys Glu Pro Val His Gly Val Ser Thr Leu Pro Glu Thr Thr Val
95 100 105

gtg agg agg acc gtg tac tat gga gtg cct gtg tgg aag tgg ctg agc 387
Val Arg Arg Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Trp Leu Ser
110 115 120

ctg ctg gtg ccc ttt gtg ggt acc 411
Leu Leu Val Pro Phe Val Gly Thr
125 130

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<210> 37
 <211> 132
 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 37
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 1 5 10 15
 Val Pro Gly Ser Arg Gly His Thr Leu Trp Lys Ala Gly Ile Leu Tyr
 20 25 30
 Lys Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Phe Leu
 35 40 45
 Pro Ser Asp Phe Phe Pro Ser Val Lys Leu Thr Pro Leu Cys Val Thr
 50 55 60
 Leu Tyr Met Asp Asp Val Val Leu Gly Ala Ser Ile Ile Asn Phe Glu
 65 70 75 80
 Lys Leu Gly Leu Ser Arg Tyr Val Ala Arg Leu Ile Leu Lys Glu Pro
 85 90 95
 Val His Gly Val Ser Thr Leu Pro Glu Thr Thr Val Val Arg Arg Thr
 100 105 110
 Val Tyr Tyr Gly Val Pro Val Trp Lys Trp Leu Ser Leu Leu Val Pro
 115 120 125
 Phe Val Gly Thr
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<210> 38
 <211> 390
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> vector pMIN.1

<221> CDS
 <222> (16)...(384)

<400> 38
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 Met Gly Met Gln Val Gln Ile Gln Ser Leu Phe Leu
 1 5 10
 ctc ctc ctg tgg gtg ccc ggg tcc aga gga cac acc ctg tgg aag gcc 99
 Leu Leu Leu Trp Val Pro Gly Ser Arg Gly His Thr Leu Trp Lys Ala
 15 20 25
 gga atc ctg tat aag gcc aag ttc gtg gct gcc tgg acc ctg aag gct 147
 Gly Ile Leu Tyr Lys Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala
 30 35 40
 gcc gct ttc ctg cct agc gat ttc ttt cct agc gtg aag ctg acc cca 195
 Ala Ala Phe Leu Pro Ser Asp Phe Phe Pro Ser Val Lys Leu Thr Pro
 45 50 55 60
 ctg tgc gtg acc ctg tat atg gat gac gtg gtg ctg gga gtg gga ctg 243
 Leu Cys Val Thr Leu Tyr Met Asp Asp Val Val Leu Gly Val Gly Leu
 65 70 75

tcc agg tac gtg gct agg ctg atc ctg aag gag cct gtg cac ggc gtg 291
 Ser Arg Tyr Val Ala Arg Leu Ile Leu Lys Glu Pro Val His Gly Val
 80 85 90

tcc acc ctg cca gag acc acc gtg gtg agg agg acc gtg tac tat gga 339
 Ser Thr Leu Pro Glu Thr Thr Val Val Arg Arg Thr Val Tyr Tyr Gly
 95 100 105

gtg cct gtg tgg aag tgg ctg agc ctg ctg gtg ccc ttt gtg 381
 Val Pro Val Trp Lys Trp Leu Ser Leu Leu Val Pro Phe Val
 110 115 120

tgaggtacc 390

<210> 39
 <211> 122
 <212> PRT
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<220>
 <223> vector pMIN.1

<400> 39
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 1 5 10 15
 Val Pro Gly Ser Arg Gly His Thr Leu Trp Lys Ala Gly Ile Leu Tyr
 20 25 30
 Lys Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Phe Leu
 35 40 45
 Pro Ser Asp Phe Phe Pro Ser Val Lys Leu Thr Pro Leu Cys Val Thr
 50 55 60
 Leu Tyr Met Asp Asp Val Val Leu Gly Val Gly Leu Ser Arg Tyr Val
 65 70 75 80
 Ala Arg Leu Ile Leu Lys Glu Pro Val His Gly Val Ser Thr Leu Pro
 85 90 95
 Glu Thr Thr Val Val Arg Arg Thr Val Tyr Tyr Gly Val Pro Val Trp
 100 105 110
 Lys Trp Leu Ser Leu Leu Val Pro Phe Val
 115 120

<210> 40
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer oligonucleotide murIi-F

<400> 40
 gctagcgccg ccaccatgga tgaccaacgc gacctc 36

<210> 41
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer oligonucleotide murIi-R

<400> 41
 ggtacctcac aggggtgactt gacccag 27

<210> 42
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer oligonucleotide IiPADRE-R

<400> 42
 cagggtccag gcagccacga acttggccac aggtttggca ga 42

<210> 43
 <211> 43
 <212> DNA
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<220>
 <223> primer oligonucleotide IiPADRE-F

<400> 43
 ggctgcttg accctgaagg ctgccgtat gtccatggat aac 43

<210> 44
 <211> 70
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligo 1

<400> 44
 cttegcata agcttatcag ccaggctgtg cagccgctc acgccgaaat caacgaagct 60
 ggaagaaccc 70

<210> 45
 <211> 70
 <212> DNA
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<220>
 <223> oligo 2

<400> 45
 ttctggtcag cagaaagaac aggataggag cgtttggagg gcgataagct ggaggggttc 60
 ttccagcttc 70

<210> 46
 <211> 71
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> oligo 3

<400> 46
 ttctgtgac cagaatcctg acaatcccc agtcctgga cgccaagttc gtggctgcct 60
 ggacctgaa g 71

<210> 47
 <211> 34
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Help-epR primer

<400> 47
 ggtacctcaa gcggcagcct tcagggtcca ggca 34

<210> 48
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> residues 323-339 of ovalbumin (Ova323-339)

<400> 48
 Ile Ser Gln Ala Val His Ala Ala His Ala Glu Ile Asn Glu Ala Gly
 1 5 10 15
 Arg

<210> 49
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> residues 128-141 of HBV core antigen (HBVcore 128)

<400> 49
 Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu
 1 5 10

<210> 50
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> residues 182-196 of HBV env (HBVenv182)

<400> 50
 Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp
 1 5 10 15

<210> 51
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Th-Pad-R primer

<400> 51
 agcggcagcc ttcagggtc 19

 <210> 52
 <211> 13
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> pan DR epitope sequence (PADRE) universal MHC
 class II epitope

 <400> 52
 Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala
 1 5 10

 <210> 53
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer Th-ova-F

 <400> 53
 atcagccagg ctgtgcacgc 20

 <210> 54
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer 1F oligonucleotide KappaSig-F

 <400> 54
 gctagcgccg ccaccatggg aatgcag 27

 <210> 55
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer 1R oligonucleotide Kappa-Th-R

 <400> 55
 cacagcctgg ctgattcctc tggaccc 27

 <210> 56
 <211> 33
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer 2F oligonucleotide PAD/LAMP-F

<400> 56
 ctgaaggctg ccgctaacaa catgttgatc ccc 33

<210> 57
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer 2R oligonucleotide LAMP-CYTOR

<400> 57
 ggtaccctag atggtctgat agcc 24

<210> 58
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer 1F oligonucleotide H2-Mb-1F

<400> 58
 gccgctagcg ccgccaccat ggtgcactc tgg 33

<210> 59
 <211> 30
 <212> DNA
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<220>
 <223> primer 1R oligonucleotide H2-Mb-1R

<400> 59
 cacagcctgg ctgatcccca tacagtgcag 30

<210> 60
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer 2F oligonucleotide H2-Mb-2F

<400> 60
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<210> 61
 <211> 24
 <212> DNA
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<220>
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<400> 61
 gcgggtaccc taatgccgtc cttc 24

<210> 62

<211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer 1F oligonucleotide H2-Ob-1F

<400> 62
 gcggctagcg ccgccaccat gggcgctggg agg 33

<210> 63
 <211> 30
 <212> DNA
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<220>
 <223> primer 1R oligonucleotide H2-Ob-1R

<400> 63
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<210> 64
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer 2F oligonucleotide H2-Ob-2F

<400> 64
 ctgaaggctg ccgctatact gaggggagct 30

<210> 65
 <211> 26
 <212> DNA
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<220>
 <223> primer 2R oligonucleotide H2-Ob-2R

<400> 65
 gccggctacct catgtgacat gtcccg 26

<210> 66
 <211> 80
 <212> DNA
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<220>
 <223> PADRE-Influenza matrix 5' primer

<400> 66
 gctagcgccg ccaccatggc caagttcgtg gctgcctgga ccctgaaggc tgccgctatg 60
 agtcttctaa ccgaggtcga 80

<210> 67
 <211> 30
 <212> DNA
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<220>
 <223> PADRE-Influenza matrix 3' primer

 <400> 67
 tcacttgaat cgctgcatct gcacccccat 30
 <210> 68
 <211> 58
 <212> DNA
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 <220>
 <223> PADRE-HBV-s one oligonucleotide

 <400> 68
 gctagcgccg ccaccatggc caagttcgtg gctgcctgga ccctgaaggc tgccgctc 58
 <210> 69
 <211> 58
 <212> DNA
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 <220>
 <223> PADRE-HBV-s second oligonucleotide

 <400> 69
 ctcgagagcg gcagccttca ggggccaggc agccacgaac ttggccatgg tggcggcg 58
 <210> 70
 <211> 33
 <212> DNA
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 <220>
 <223> primer 1F oligonucleotide Ig alpha-1F

 <400> 70
 gcggctagcg ccgccaccat gccaggggggt cta 33
 <210> 71
 <211> 30
 <212> DNA
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 <220>
 <223> primer 1R oligonucleotide Ig alpha-1R

 <400> 71
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 <210> 72
 <211> 30
 <212> DNA
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 <220>
 <223> primer 2F oligonucleotide Ig alpha-2F

<400> 72
 ctgaaggctg ccgctgggat catcttgctg 30

 <210> 73
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer 2R oligonucleotide Ig alpha-2R

 <400> 73
 gcgggtacct catggctttt ccagctg 27

 <210> 74
 <211> 33
 <212> DNA
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 <220>
 <223> primer 1F oligonucleotide B29-1F

 <400> 74
 gcggctagcg ccgccaccat ggccacactg gtg 33

 <210> 75
 <211> 30
 <212> DNA
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 <220>
 <223> primer 1R oligonucleotide B29-1R

 <400> 75
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 <210> 76
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer 2F oligonucleotide B29-2F (30mer)

 <400> 76
 ctgaaggctg ccgctattat cttgatccag 30

 <210> 77
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> primer 2R oligonucleotide B29-2R (27mer)

 <400> 77
 gccggtacct cattcctggc ctggatg 27

 <210> 78

<211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> pUC4K amplification primer

 <400> 78
 tctgatgtta cattgcacaa g 21

 <210> 79
 <211> 33
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> pUC4K amplification primer

 <400> 79
 gcgcactcat gatgctctgc cagtgttaca acc 33

 <210> 80
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> CMV DNA amplification primer

 <400> 80
 gcgtctagag taagtaccgc ctatagactc 30

 <210> 81
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> CMV DNA amplification primer

 <400> 81
 ccggctagcc tgcagaaaag acccatggaa 30

 <210> 82
 <211> 49
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> polylinker oligo

 <400> 82
 ggccgcaagg aaaaaatcta gagtcggcca tagactaatg ccggtaccg 49

 <210> 83
 <211> 50
 <212> DNA
 <213> Artificial Sequence

<220>

<223> polylinker oligo

<400> 83

ctagcgggtac cggcattagt ctatggcccg actctagatt ttttccttgc

50

<210> 84

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> oligo annealed to produce fragment with
polylinker, polyadenylation signal and EcoRI and
XhoI cohesive ends

<400> 84

aattcggata tccaagcttg atgaataaaa gatcagagct ctagtgatct gtgtgttggt
ttttttgtgt gc

60

72

<210> 85

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> oligo annealed to produce fragment with
polylinker, polyadenylation signal and EcoRI and
XhoI cohesive ends

<400> 85

tcgagcacac aaaaaaccaa cacacagatc actagagctc tgatcttttt attcatcaag
cttgatatac cg

60

72

<210> 86

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> consensus mouse Ig Kappa signal sequence

<400> 86

Met Gln Val Gln Ile Gln Ser Leu Phe Leu Leu Leu Leu Trp Val Pro
1 5 10 15
Gly Ser Arg Gly
20

<210> 87

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> nucleotides encoding consensus mouse Ig Kappa
signal sequence

<400> 87
 atgcaggtgc agatccagag cctgtttctg ctctctctgt gggcgcccggtgccagagga 60

<210> 88
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 149-159 (All restricted) (peptide 1147.16)

<400> 88
 His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys
 1 5 10

<210> 89
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding HBV pol 149-159 (All
 restricted)

<400> 89
 cacaccctgt ggaaggccgg aatcctgtat aag 33

<210> 90
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding PADRE-universal MHC class II
 epitope

<400> 90
 gccaaagtctg tggctgctg gaccctgaag gctgccgct 39

<210> 91
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV core 18-27 (A2 restricted) (peptide 924.07)

<400> 91
 Phe Leu Pro Ser Asp Phe Phe Pro Ser Val
 1 5 10

<210> 92
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding HBV core 18-27 (A2
 restricted)

79tt

<400> 92
 ttccctgccta gcgatttctt tccctagcgtg 30

 <210> 93
 <211> 9
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HIV env 120-128 (A2 restricted) (peptide 1211.04)

 <400> 93
 Lys Leu Thr Pro Leu Cys Val Thr Leu
 1 5

 <210> 94
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> nucleotides encoding HIV env 120-128 (A2
 restricted)

 <400> 94
 aagctgaccc cactgtgcgt gaccctg 27

 <210> 95
 <211> 9
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HBV pol 551-559 (A2 restricted) (peptide 1090.14)

 <400> 95
 Tyr Met Asp Asp Val Val Leu Gly Ala
 1 5

 <210> 96
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> nucleotides encoding HBV pol 551-559 (A2
 restricted)

 <400> 96
 tatatggatg acgtggtgct gggagcc 27

 <210> 97
 <211> 8
 <212> PRT
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 <220>
 <223> mouse ovalbumin 257-264 (Kb restricted)

<400> 97
 Ser Ile Ile Asn Phe Glu Lys Leu
 1 5

<210> 98
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding mouse ovalbumin 257-264 (Kb
 restricted)

<400> 98
 agcatcatca acttogagaa gctg

24

<210> 99
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 455-463 (A2 restricted) (peptide 1168.02)

<400> 99
 Gly Leu Ser Arg Tyr Val Ala Arg Leu
 1 5

<210> 100
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding HBV pol 455-463 (A2
 restricted)

<400> 100
 ggactgttca gatacgtggc taggctg

27

<210> 101
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV pol 476-484 (A2 restricted) (peptide 941.031)

<400> 101
 Ile Leu Lys Glu Pro Val His Gly Val
 1 5

<210> 102
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding HIV pol 476-484 (A2 restricted)

 <400> 102
 atcctgaagg agcctgtgca cggcgtg 27

 <210> 103
 <211> 11
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HBV core 141-151 (All restricted) (peptide 1083.01)

 <400> 103
 Ser Thr Leu Pro Glu Thr Thr Val Val Arg Arg
 1 5 10
 <210> 104
 <211> 33
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> nucleotides encoding HBV core 141-151 (All restricted)

 <400> 104
 tccaccctgc cagagaccac cgtgggtgagg aga 33

 <210> 105
 <211> 10
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HIV env 49-58 (All restricted) (peptide 1069.43)

 <400> 105
 Thr Val Tyr Tyr Gly Val Pro Val Trp Lys
 1 5 10
 <210> 106
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> nucleotides encoding HIV env 49-58 (All restricted)

 <400> 106
 accgtgtact atggagtgcc tgtgtggaag 30

 <210> 107
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBVadr-ENV (S Ag 335-343) (A2 restricted) (peptide
 1013.0102)

<400> 107
 Trp Leu Ser Leu Leu Val Pro Phe Val
 1 5

<210> 108
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> nucleotides encoding HBV env 335-343 (A2
 restricted)

<400> 108
 tggctgagcc tgctggtgcc ctttgtg 27

<210> 109
 <211> 12
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> consensus Kozak sequence

<400> 109
 gccgccacca tg 12

<210> 110
 <211> 70
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Min1 oligo

<400> 110
 gaggagcaga aacaggctct ggatctgcac ctgcattccc atggtggcgg cgctagcaag 60
 cttcttgccg 70

<210> 111
 <211> 71
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Min2 oligo

<400> 111
 cctgtttctg ctctctctgt gggtgcccgg gtccagagga cacaccctgt ggaaggccgg 60
 aatctgtat a 71

<210> 112
 <211> 70
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Min3 oligo

<400> 112

tcgctaggca ggaaagcggc agccttcagg gtccaggcag ccacgaactt ggccttatac 60
aggattccgg 70

<210> 113

<211> 71

<212> DNA

<213> Artificial Sequence

<220>

<223> Min4 oligo

<400> 113

ctttcctgcc tagcgatttc tttcctagcg tgaagctgac ccactgtgc gtgaccctgt 60
atatggatga c 71

<210> 114

<211> 70

<212> DNA

<213> Artificial Sequence

<220>

<223> Min5 oligo

<400> 114

cgtacctgga cagtcccagc ttctcgaagt tgatgatgct ggctcccagc accacgtcat 60
ccatatacag 70

<210> 115

<211> 71

<212> DNA

<213> Artificial Sequence

<220>

<223> Min6 oligo

<400> 115

ggactgtcca gatacgtggc taggctgac ctgaaggagc ctgtgcacgg cgtgtccacc 60
ctgccagaga c 71

<210> 116

<211> 70

<212> DNA

<213> Artificial Sequence

<220>

<223> Min7 oligo

<400> 116

gctcagccac ttccacacag gcactccata gtacacggtc ctctccacca cggtggtctc 60
tggcagggtg 70

<210> 117

<211> 51

<212> DNA

<213> Artificial Sequence

79yy

<220>
 <223> Min8 oligo

<400> 117
 gtggaagtgg ctgagcctgc tgggtgccctt tgtgggtacc tgatctagag c 51

<210> 118
 <211> 20
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<220>
 <223> flanking primer 5'

<400> 118
 gcgcaagaag cttgctagcg 20

<210> 119
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> flanking primer 3'

<400> 119
 gctctagatc aggtacccca c 21

<210> 120
 <211> 30
 <212> DNA
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<220>
 <223> primer Min-ovaR

<400> 120
 tggacagtcc cactcccagc accacgtcat 30

<210> 121
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 <212> DNA
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<220>
 <223> primer Min-ovaF

<400> 121
 gctgggagtg ggactgtcca ggtacgtggc 30

<210> 122
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer Min-StopR

<400> 122
 ggtacctcac acaaagggca ccagcaggc

29

<210> 123
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV Env-HIV Pol 476

<400> 123
 Leu Leu Val Pro Phe Val Ile Leu
 1 5

<210> 124
 <211> 14
 <212> PRT
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<220>
 <223> HBVcore128

<400> 124
 Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Phe
 1 5 10

<210> 125
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV Pol 551-V (peptide 1090.77)

<400> 125
 Tyr Met Asp Asp Val Val Leu Gly Val
 1 5

<210> 126
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> first reaction amplification primer for pMin.1-No
 PADRE

<400> 126
 atcgctaggc aggaacttat acaggattcc

30

<210> 127
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> first reaction amplification primer for
 pMin.1-Anchor

79aaa

<400> 127
 tggacagtcc ggctcccagc accacgt 27

 <210> 128
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> 3' amplification primer (No PADRE)

 <400> 128
 ttctctgccta gcgatttc 18

 <210> 129
 <211> 27
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> 3' amplification primer (Anchor)
 <400> 129
 gctgggagcc ggactgtcca ggtacgt 27

 <210> 130
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> PCR amplification primer for Ig signal sequence
 deletion from pMin.1 for pMin.1-No Sig

 <400> 130
 gctagcgccg ccaccatgca caccctgtgg aaggccggaa tc 42

 <210> 131
 <211> 39
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> pMin.1-Switch 5' fragment amplification primer

 <400> 131
 gggcaccagc aggctcagcc acactcccag caccacgtc 39

 <210> 132
 <211> 40
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> pMin.1-Switch second overlapping fragment
 amplification primer

 <400> 132
 agcctgctgg tgcccttctg gatcctgaag gagcctgtgc 40

 <210> 133

<211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> pMin.1-Switch second overlapping fragment
 amplification primer

 <400> 133
 agccacgtac ctggacagtc ccttcacac aggcactcca t 41

 <210> 134
 <211> 32
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> pMin.1-Switch 3' third fragment amplification
 primer

 <400> 134
 tgtccaggta cgtggctagg ctgtgaggta cc 32
 <210> 135
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> PCR amplification primer Min.0-No Sig-5' for
 deletion of signal sequence from pMin.0 for
 pMin.2-GFP

 <400> 135
 gctagcgccg ccaccatgca caccctgtgg aaggccggaa tc 42

 <210> 136
 <211> 10
 <212> PRT
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 <220>
 <223> HBV Env 335-HBV Pol 551

 <400> 136
 Val Leu Gly Val Trp Leu Ser Leu Leu Val
 1 5 10

 <210> 137
 <211> 15
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HBV POL 661 (peptide 1298.06)

 <400> 137
 Lys Gln Ala Phe Thr Phe Ser Pro Thr Tyr Lys Ala Phe Leu Cys
 1 5 10 15

 <210> 138
 79ccc

<211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 412 (peptide F107.03)

<400> 138
 Leu Gln Ser Leu Thr Asn Leu Leu Ser Ser Asn Leu Ser Trp Leu
 1 5 10 15

<210> 139
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ENV 180 (peptide 1280.06)

<400> 139
 Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser
 1 5 10 15

<210> 140
 <211> 15
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<220>
 <223> HBV POL 774 (peptide 1280.09)

<400> 140
 Gly Thr Ser Phe Val Tyr Val Pro Ser Ala Leu Asn Pro Ala Asp
 1 5 10 15

<210> 141
 <211> 20
 <212> PRT
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<220>
 <223> HBV NUC 120 (peptide CF-08)

<400> 141
 Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr Arg Pro Pro
 1 5 10 15
 Asn Ala Pro Ile
 20

<210> 142
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV NUC 123 (peptide 27.0280)

<400> 142
 Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr Arg Pro Pro Asn Ala
 1 5 10 15

<210> 143
 <211> 15
 <212> PRT
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<220>
 <223> HBV NUC 121 (peptide 1186.25)

<400> 143
 Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr Arg Pro Pro
 1 5 10 15

<210> 144
 <211> 15
 <212> PRT
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<220>
 <223> HBV POL 145 (peptide 27.0281)

<400> 144
 Arg His Tyr Leu His Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys
 1 5 10 15

<210> 145
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 523 (peptide F107.04)

<400> 145
 Pro Phe Leu Leu Ala Gln Phe Thr Ser Ala Ile Cys Ser Val Val
 1 5 10 15

<210> 146
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ENV 339 (peptide 1186.15)

<400> 146
 Leu Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val
 1 5 10 15

<210> 147
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 501 (peptide 1280.15)

<400> 147

79eee

Leu His Leu Tyr Ser His Pro Ile Ile Leu Gly Phe Arg Lys Ile
 1 5 10 15

<210> 148
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 615 (peptide 1298.04)

<400> 148
 Lys Gln Cys Phe Arg Lys Leu Pro Val Asn Arg Pro Ile Asp Trp
 1 5 10 15

<210> 149
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 764 (peptide 1298.07)

<400> 149
 Ala Ala Asn Trp Ile Leu Arg Gly Thr Ser Phe Val Tyr Val Pro
 1 5 10 15

<210> 150
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV CORE 50 (peptide 857.02)

<400> 150
 Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu
 1 5 10 15
 Met Thr Leu Ala
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<210> 151
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 683 (peptide 35.0100)

<400> 151
 Leu Cys Gln Val Phe Ala Asp Ala Thr Pro Thr Gly Trp Gly Leu
 1 5 10 15

<210> 152
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

79fff

<223> HBV POL 387 (peptide 35.0096)

<400> 152

Glu	Ser	Arg	Leu	Val	Val	Asp	Phe	Ser	Gln	Phe	Ser	Arg	Gly	Asn
1				5					10					15

<210> 153

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 96 (peptide 35.0093)

<400> 153

Val	Gly	Pro	Leu	Thr	Val	Asn	Glu	Lys	Arg	Arg	Leu	Lys	Leu	Ile
1				5					10					15

<210> 154

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 422 (peptide 1186.18)

<400> 154

Asn	Leu	Ser	Trp	Leu	Ser	Leu	Asp	Val	Ser	Ala	Ala	Phe	Tyr	His
1				5					10					15

<210> 155

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV ayw 183 (peptide 777.03)

<400> 155

Phe	Leu	Leu	Thr	Arg	Ile	Leu	Thr	Ile
1				5				

<210> 156

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ayw pol 642 (peptide 927.15)

<400> 156

Ala	Leu	Met	Pro	Leu	Tyr	Ala	Cys	Ile
1				5				

<210> 157

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 562 (peptide 927.11)

<400> 157

Phe	Leu	Leu	Ser	Leu	Gly	Ile	His	Leu
1				5				

<210> 158

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 531 (peptide 1090.11)

<400> 158

Ser	Ala	Ile	Cys	Ser	Val	Val	Arg	Arg
1				5				

<210> 159

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 665 (peptide 1090.10)

<400> 159

Gln	Ala	Phe	Thr	Phe	Ser	Pro	Thr	Tyr	Lys
1				5				10	

<210> 160

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 47 (peptide 1069.16)

<400> 160

Asn	Val	Ser	Ile	Pro	Trp	Thr	His	Lys
1				5				

<210> 161

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 388 (peptide 1069.20)

<400> 161

Leu	Val	Val	Asp	Phe	Ser	Gln	Phe	Ser	Arg
1				5				10	

<210> 162

<211> 9

<212> PRT

<213> Artificial Sequence

79hhh

<220>

<223> HBV adr POL 629 (peptide 1142.05) (peptide 1.0166)

<400> 162

Lys Val Gly Asn Phe Thr Gly Leu Tyr

1

5

<210> 163

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 150 (peptide 1069.15)

<400> 163

Thr Leu Trp Lys Ala Gly Ile Leu Tyr Lys

1

5

10

<210> 164

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 313 (peptide 1145.04)

<400> 164

Ile Pro Ile Pro Ser Ser Trp Ala Phe

1

5

<210> 165

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV core 19-27 (peptide 988.05)

<400> 165

Leu Pro Ser Asp Phe Phe Pro Ser Val

1

5

<210> 166

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 354 (peptide 1147.04)

<400> 166

Thr Pro Ala Arg Val Thr Gly Gly Val Phe

1

5

10

<210> 167

<211> 10

<212> PRT

79iii

<213> Artificial Sequence

<220>

<223> HBV env 338-347 (peptide 1069.06)

<400> 167

Leu	Leu	Val	Pro	Phe	Val	Gln	Trp	Phe	Val
1				5					10

<210> 168

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 513 (peptide 1147.13)

<400> 168

Phe	Leu	Leu	Ala	Gln	Phe	Thr	Ser	Ala	Ile
1				5					10

<210> 169

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 259 (peptide 1147.14)

<400> 169

Val	Leu	Leu	Asp	Tyr	Gln	Gly	Met	Leu	Pro	Val
1				5						10

<210> 170

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 339 (peptide 1132.01)

<400> 170

Leu	Val	Pro	Phe	Val	Gln	Trp	Phe	Val
1				5				

<210> 171

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 504-512 (peptide1069.05)

<400> 171

Leu	Leu	Ala	Gln	Phe	Thr	Ser	Ala	Ile
1				5				

<210> 172

<211> 9

79jjj

<212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 411 (peptide 927.42)

<400> 172
 Asn Leu Ser Trp Leu Ser Leu Asp Val
 1 5

<210> 173
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 992 (peptide 927.41)

<400> 173
 Leu Leu Ser Ser Asn Leu Ser Trp Leu
 1 5

<210> 174
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 489 (peptide 927.46)

<400> 174
 Lys Leu His Leu Tyr Ser His Pro Ile
 1 5

<210> 175
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 503 (peptide 1069.071)

<400> 175
 Phe Leu Leu Ala Gln Phe Thr Ser Ala
 1 5

<210> 176
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ENV 62 (peptide 1142.07)

<400> 176
 Gly Leu Leu Gly Trp Ser Pro Gln Ala
 1 5

<210> 177

79kkk

<211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ayw pol 1076 (peptide 927.47)

<400> 177
 His Leu Tyr Ser His Pro Ile Ile Leu
 1 5

<210> 178
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV env 377-385 (peptide 1069.13)

<400> 178
 Pro Leu Leu Pro Ile Phe Phe Cys Leu
 1 5

<210> 179
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV adr-ENV 177 (peptide 1013.1402)

<400> 179
 Val Leu Gln Ala Gly Phe Phe Leu Leu
 1 5

<210> 180
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 376 (peptide 26.0539)

<400> 180
 Arg Leu Val Val Asp Phe Ser Gln Phe Ser Arg
 1 5 10

<210> 181
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV X nuc fus 299 (peptide 26.0535)

<400> 181
 Gly Val Trp Ile Arg Thr Pro Pro Ala Tyr Arg
 1 5 10

<210> 182
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV X 64 (peptide 26.0153)

<400> 182
 Ser Ser Ala Gly Pro Cys Ala Leu Arg
 1 5

<210> 183
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV adr "X" 1548 (peptide 1.0993)

<400> 183
 Lys Val Phe Val Leu Gly Gly Cys Arg
 1 5

<210> 184
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV X 69 (peptide 26.0149)

<400> 184
 Cys Ala Leu Arg Phe Thr Ser Ala Arg
 1 5

<210> 185
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV x nuc fus 296 (peptide 26.0023)

<400> 185
 Val Ser Phe Gly Val Trp Ile Arg
 1 5

<210> 186
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV x nuc fus 318 (peptide 26.0545)

<400> 186
 Thr Leu Pro Glu Thr Thr Val Val Arg Arg Arg
 1 5 10

<210> 187
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 524 (peptide 20.0131)

<400> 187
 Ser Val Val Arg Arg Ala Phe Pro His
 1 5

<210> 188
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV adr "X" 1550 (peptide 1.0219)

<400> 188
 Phe Val Leu Gly Gly Cys Arg His Lys
 1 5

<210> 189
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 656 (peptide 26.0008)

<400> 189
 Phe Thr Phe Ser Pro Thr Tyr Lys
 1 5

<210> 190
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 655 (peptide 20.0130)

<400> 190
 Ala Phe Thr Glu Ser Pro Thr Tyr Lys
 1 5

<210> 191
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>

<223> HBV POL 530 (peptide 1147.05)

<400> 191

Phe	Pro	His	Cys	Leu	Ala	Phe	Ser	Tyr	Met
1				5					10

<210> 192

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 640 (peptide 1147.08)

<400> 192

Tyr	Pro	Ala	Leu	Met	Pro	Leu	Tyr	Ala
1				5				

<210> 193

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV X 58 (peptide 1147.06)

<400> 193

Leu	Pro	Val	Cys	Ala	Phe	Ser	Ser	Ala
1				5				

<210> 194

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 429 (peptide 1147.02)

<400> 194

His	Pro	Ala	Ala	Met	Pro	His	Leu	Leu
1				5				

<210> 195

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 640 (peptide 26.0570)

<400> 195

Tyr	Pro	Ala	Leu	Met	Pro	Leu	Tyr	Ala	Cys	Ile
1				5					10	

<210> 196

<211> 8

<212> PRT

<213> Artificial Sequence

79000

<220>

<223> HBV POL 640 (peptide 19.0014)

<400> 196

Tyr Pro Ala Leu Met Pro Leu Tyr

1

5

<210> 197

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 541 (peptide 1145.08)

<400> 197

Phe Pro His Cys Leu Ala Phe Ser Tyr

1

5

<210> 198

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV NUC 131 (peptide 1090.02)

<400> 198

Ala Tyr Arg Pro Pro Asn Ala Pro Ile

1

5

<210> 199

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV adr CORE 419 (peptide 1.0519)

<400> 199

Asp Leu Leu Asp Thr Ala Ser Ala Leu Tyr

1

5

10

<210> 200

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV NUC 117 (peptide 13.0129)

<400> 200

Glu Tyr Leu Val Ser Phe Gly Val Trp Ile

1

5

10

<210> 201

<211> 10

<212> PRT

79ppp

<213> Artificial Sequence

<220>

<223> HBV POL 631 (peptide 20.0254)

<400> 201

Phe	Ala	Ala	Pro	Phe	Thr	Gln	Cys	Gly	Tyr
1				5				10	

<210> 202

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ALL 1224 (peptide 2.0060)

<400> 202

Gly	Tyr	Pro	Ala	Leu	Met	Pro	Leu	Tyr
1				5				

<210> 203

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV pol 149 (peptide 1069.04)

<400> 203

His	Thr	Leu	Trp	Lys	Ala	Gly	Ile	Leu	Tyr
1				5				10	

<210> 204

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV env 249-258 (peptide 1069.08)

<400> 204

Ile	Leu	Leu	Leu	Cys	Leu	Ile	Phe	Leu	Leu
1				5				10	

<210> 205

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 745 (peptide 1069.23)

<400> 205

Lys	Tyr	Thr	Ser	Phe	Pro	Trp	Leu	Leu
1				5				

<210> 206

<211> 9

79qqq

<212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV core 59 (peptide 1069.01)

<400> 206
 Leu Leu Asp Thr Ala Ser Ala Leu Tyr
 1 5

<210> 207
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ALL 1000 (peptide 2.0239)

<400> 207
 Leu Ser Leu Asp Val Ser Ala Ala Phe Tyr
 1 5 10

<210> 208
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 492 (peptide 2.0181)

<400> 208
 Leu Tyr Ser His Pro Ile Ile Leu Gly Phe
 1 5 10

<210> 209
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV 360 (peptide 1039.01)

<400> 209
 Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr
 1 5 10

<210> 210
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV adr 1521 (peptide 2.0126)

<400> 210
 Met Ser Thr Thr Asp Leu Glu Ala Tyr
 1 5

79rrr

<210> 211
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 124 (peptide 1069.03)

<400> 211
 Pro Leu Asp Lys Gly Ile Lys Pro Tyr Tyr
 1 5 10

<210> 212
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 808 (peptide 1090.09)

<400> 212
 Pro Thr Thr Gly Arg Thr Ser Leu Tyr
 1 5

<210> 213
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 51 (peptide 20.0138)

<400> 213
 Pro Trp Thr His Lys Val Gly Asn Phe
 1 5

<210> 214
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ENV 236 (peptide 20.0135)

<400> 214
 Arg Trp Met Cys Leu Arg Arg Phe Ile
 1 5

<210> 215
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ENV 236 (peptide 20.0269)

<400> 215
 Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
 1 5 10

<210> 216
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 167 (peptide 20.0139)

<400> 216
 Ser Phe Cys Gly Ser Pro Tyr Ser Trp
 1 5

<210> 217
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV pol 427 (peptide 1069.02)

<400> 217
 Ser Leu Asp Val Ser Ala Ala Phe Tyr
 1 5

<210> 218
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV ENV 334 (peptide 20.0136)

<400> 218
 Ser Trp Leu Ser Leu Leu Val Pro Phe
 1 5

<210> 219
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 392 (peptide 20.0271)

<400> 219
 Ser Trp Pro Lys Phe Ala Val Pro Asn Leu
 1 5 10

<210> 220
 <211> 9
 <212> PRT
 <213> Artificial Sequence

79ttt

<220>

<223> HBV ENV 197 (peptide 20.0137)

<400> 220

Ser	Trp	Trp	Thr	Ser	Leu	Asn	Phe	Leu
1				5				

<210> 221

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 4 (peptide 2.0173)

<400> 221

Ser	Tyr	Gln	His	Phe	Arg	Lys	Leu	Leu	Leu
1				5				10	

<210> 222

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV NUC 102 (peptide 13.0073)

<400> 222

Trp	Phe	His	Ile	Ser	Cys	Leu	Thr	Phe
1				5				

<210> 223

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV adr CORE 416 (peptide 1.0774)

<400> 223

Trp	Leu	Trp	Gly	Met	Asp	Ile	Asp	Pro	Tyr
1				5					10

<210> 224

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV env 359 (peptide 1039.06)

<400> 224

Trp	Met	Met	Trp	Tyr	Trp	Gly	Pro	Ser	Leu	Tyr
1				5					10	

<210> 225

<211> 10

<212> PRT

<213> Artificial Sequence

79uuu

<220>

<223> HBv 18-27 I10 var. (peptide 924.14)

<400> 225

Phe	Leu	Pro	Ser	Asp	Phe	Phe	Pro	Ser	Ile
1				5					10

<210> 226

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HBc 18-27 analog (peptide 941.01)

<400> 226

Phe	Leu	Pro	Ser	Asp	Tyr	Phe	Pro	Ser	Val
1				5					10

<210> 227

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV core 141-151 analog (peptide 1083.02)

<400> 227

Ser	Thr	Leu	Pro	Glu	Thr	Tyr	Val	Val	Arg	Arg
1				5						10

<210> 228

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 313 analog (peptide 1145.05)

<400> 228

Phe	Pro	Ile	Pro	Ser	Ser	Trp	Ala	Phe
1				5				

<210> 229

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 541 analog (peptide 1145.11)

<400> 229

Phe	Pro	His	Cys	Leu	Ala	Phe	Ser	Leu
1				5				

<210> 230

<211> 9

<212> PRT

79vvv

<213> Artificial Sequence

<220>

<223> HBV POL 541 analog (peptide 1145.24)

<400> 230

Phe Pro His Cys Leu Ala Phe Ala Leu
1 5

<210> 231

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 313 analog (peptide 1145.06)

<400> 231

Ile Pro Ile Thr Ser Ser Trp Ala Phe
1 5

<210> 232

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 313 analog (peptide 1145.23)

<400> 232

Ile Pro Ile Pro Met Ser Trp Ala Phe
1 5

<210> 233

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV ENV 313 analog (peptide 1145.07)

<400> 233

Ile Pro Ile Leu Ser Ser Trp Ala Phe
1 5

<210> 234

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HBV POL 541 analog (peptide 1145.09)

<400> 234

Phe Pro Val Cys Leu Ala Phe Ser Tyr
1 5

<210> 235

<211> 9

<212> PRT
 <213> Artificial Sequence

<220>
 <223> HBV POL 541 analog (peptide 1145.10)

<400> 235
 Phe Pro His Cys Leu Ala Phe Ala Tyr
 1 5

<210> 236
 <211> 26
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1242-1267

<400> 236
 Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser
 1 5 10 15
 Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr
 20 25

<210> 237
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1242 (peptide P98.03)

<400> 237
 Ala Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser
 1 5 10 15
 Val Ala Ala Thr
 20

<210> 238
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1248 (peptide P98.04)

<400> 238
 Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly
 1 5 10 15
 Phe Gly Ala Tyr
 20

<210> 239
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>

<223> HCV NS3 1248 (peptide P98.05)

<400> 239

Gly	Tyr	Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala	Thr
1				5					10				

<210> 240

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS3 1253 (peptide 1283.21)

<400> 240

Gly	Tyr	Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala	Thr	Leu
1				5					10					15

<210> 241

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS3 1251 (peptide 1283.20)

<400> 241

Ala	Gln	Gly	Tyr	Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala
1				5					10					15

<210> 242

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS4 1914-1935

<400> 242

Gly	Glu	Gly	Ala	Val	Gln	Trp	Met	Asn	Arg	Leu	Ile	Ala	Phe	Ala	Ser
1				5					10					15	
Arg	Gly	Asn	His	Val	Ser										
				20											

<210> 243

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS4 1914 (peptide F134.08)

<400> 243

Gly	Glu	Gly	Ala	Val	Gln	Trp	Met	Asn	Arg	Leu	Ile	Ala	Phe	Ala	Ser
1				5					10					15	
Arg	Gly	Asn	His	Val											
				20											

<210> 244

79yyy

<211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1921 (peptide 1283.44)

<400> 244
 Met Asn Arg Leu Ile Ala Phe Ala Ser Arg Gly Asn His Val Ser
 1 5 10 15

<210> 245
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1025 (peptide 1283.16)

<400> 245
 Ser Lys Gly Trp Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln
 1 5 10 15

<210> 246
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 2641 (peptide 1283.55)

<400> 246
 Gly Ser Ser Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu
 1 5 10 15

<210> 247
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1772 (peptide F134.05)

<400> 247
 Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro
 1 5 10 15
 Gly Asn Pro Ala
 20

<210> 248
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 2939 (peptide 1283.61)

<400> 248

Ala	Ser	Cys	Leu	Arg	Lys	Leu	Gly	Val	Pro	Pro	Leu	Arg	Val	Trp
1				5					10					15

<210> 249

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS3 1393 (peptide 1283.25)

<400> 249

Gly	Arg	His	Leu	Ile	Phe	Cys	His	Ser	Lys	Lys	Lys	Cys	Asp	Glu
1				5					10					15

<210> 250

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV 1466 (peptide 35.0107)

<400> 250

Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe	Thr	Ile	Glu	Thr	Thr
1				5					10					15

<210> 251

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV 1437 (peptide 35.0106)

<400> 251

Val	Val	Val	Val	Ala	Thr	Asp	Ala	Leu	Met	Thr	Gly	Tyr	Thr	Gly
1				5					10					15

<210> 252

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS1/E2 728 (peptide 1090.18)

<400> 252

Phe	Leu	Leu	Leu	Ala	Asp	Ala	Arg	Val
1				5				

<210> 253

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS4 1812 (peptide 1073.05)

<400> 253

79aaaa

Leu Leu Phe Asn Ile Leu Gly Gly Trp Val
 1 5 10

<210> 254
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1590 (peptide 1013.02)

<400> 254

Tyr Leu Val Ala Tyr Gln Ala Thr Val
 1 5

<210> 255
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 132 (peptide 1013.1002)

<400> 255

Asp Leu Met Gly Tyr Ile Pro Leu Val
 1 5

<210> 256
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 2611 (peptide 1090.22)

<400> 256

Arg Leu Ile Val Phe Pro Asp Leu Gly Val
 1 5 10

<210> 257
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1666 (peptide 24.0075)

<400> 257

Val Leu Val Gly Gly Val Leu Ala Ala
 1 5

<210> 258
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1920 (peptide 24.0073)

79bbbb

<400> 258
 Trp Met Asn Arg Leu Ile Ala Phe Ala
 1 5

<210> 259
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1769 (peptide 1174.08)

<400> 259
 His Met Trp Asn Phe Ile Ser Gly Ile
 1 5

<210> 260
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1851 (peptide 1073.06)

<400> 260
 Ile Leu Ala Gly Tyr Gly Ala Gly Val
 1 5

<210> 261
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS1/E2 726 (peptide 24.0071)

<400> 261
 Leu Leu Phe Leu Leu Leu Ala Asp Ala
 1 5

<210> 262
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 35 (peptide 1073.07)

<400> 262
 Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu
 1 5 10

<210> 263
 <211> 9
 <212> PRT
 <213> Artificial Sequence

79cccc

<220>
 <223> HCV NS3 1136 (peptide 1.0119)

 <400> 263
 Tyr Leu Val Thr Arg His Ala Asp Val
 1 5

 <210> 264
 <211> 9
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HCV Core 51 (peptide 1.0952)

 <400> 264
 Lys Thr Ser Glu Arg Ser Gln Pro Arg
 1 5

 <210> 265
 <211> 10
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HCV NS4 1863 (peptide 1073.10)

 <400> 265
 Gly Val Ala Gly Ala Leu Val Ala Phe Lys
 1 5 10

 <210> 266
 <211> 9
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HCV NS3 1391 (peptide 1.0123)

 <400> 266
 Leu Ile Phe Cys His Ser Lys Lys Lys
 1 5

 <210> 267
 <211> 9
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> HCV E1 290 (peptide 1.0955)

 <400> 267
 Gln Leu Phe Thr Phe Ser Pro Arg Arg
 1 5

 <210> 268
 <211> 9
 <212> PRT
 <213> Artificial Sequence
 79dddd

<220>
 <223> HCV Core 43 (peptide 1073.11)

<400> 268
 Arg Leu Gly Val Arg Ala Thr Arg Lys
 1 5

<210> 269
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS1/E2 635 (peptide 1073.13)

<400> 269
 Arg Met Tyr Val Gly Gly Val Glu His Arg
 1 5 10

<210> 270
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1864 (peptide 24.0090)

<400> 270
 Val Ala Gly Ala Leu Val Ala Phe Lys
 1 5

<210> 271
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 3036 (peptide F104.01)

<400> 271
 Val Gly Ile Tyr Leu Leu Pro Asn Arg
 1 5

<210> 272
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 168 (peptide 1145.12)

<400> 272
 Leu Pro Gly Cys Ser Phe Ser Ile Phe
 1 5

<210> 273
 <211> 8
 <212> PRT

79eeee

<213> Artificial Sequence

<220>

<223> HCV 1378 (peptide 29.0035)

<400> 273

Ile Pro Phe Tyr Gly Lys Ala Ile
1 5

<210> 274

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS3 1128 (peptide 1069.62)

<400> 274

Cys Thr Cys Gly Ser Ser Asp Leu Tyr
1 5

<210> 275

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS4 1765 (peptide 24.0092)

<400> 275

Phe Trp Ala Lys His Met Trp Asn Phe
1 5

<210> 276

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS5 2922 (peptide 13.0019)

<400> 276

Leu Ser Ala Phe Ser Leu His Ser Tyr
1 5

<210> 277

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HCV NS3 1267 (peptide 24.0086)

<400> 277

Leu Gly Phe Gly Ala Tyr Met Ser Lys
1 5

<210> 278

<211> 9

79ffff

<212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 2621 (peptide 1174.21)

<400> 278
 Arg Val Cys Glu Lys Met Ala Leu Tyr
 1 5

<210> 279
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS1/E2 557 (peptide 1174.16)

<400> 279
 Trp Met Asn Ser Thr Gly Phe Thr Lys
 1 5

<210> 280
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1622 (peptide 1073.04)

<400> 280
 Thr Leu His Gly Pro Thr Pro Leu Leu Tyr
 1 5 10

<210> 281
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS3 1588 (peptide 16.0012)

<400> 281
 Phe Pro Tyr Leu Val Ala Tyr Gln Ala
 1 5

<210> 282
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS1/E2 623 (peptide 15.0047)

<400> 282
 Tyr Pro Cys Thr Val Asn Phe Thr Ile
 1 5

<210> 283

79gggg

<211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 2129 (peptide 24.0093)

<400> 283
 Glu Val Asp Gly Val Arg Leu His Arg Tyr
 1 5 10

<210> 284
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV 126 (peptide 3.0417)

<400> 284
 Leu Thr Cys Gly Phe Ala Asp Leu Met Gly Tyr
 1 5 10

<210> 285
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV E1 700 (peptide 1073.01)

<400> 285
 Asn Ile Val Asp Val Gln Tyr Leu Tyr
 1 5

<210> 286
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS5 2921 (peptide 1.0509)

<400> 286
 Gly Leu Ser Ala Phe Ser Leu His Ser Tyr
 1 5 10

<210> 287
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV E1 275 (peptide 1073.17)

<400> 287
 Met Tyr Val Gly Asp Leu Cys Gly Ser Val Phe
 1 5 10

79hhhh

<210> 288
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS1/E2 633 (peptide 1073.18)

<400> 288
 Met Tyr Val Gly Gly Val Glu His Arg Leu
 1 5 10

<210> 289
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV NS4 1778 (peptide 13.075)

<400> 289
 Gln Tyr Leu Ala Gly Leu Ser Thr Leu
 1 5

<210> 290
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 168 (peptide 1145.13)

<400> 290
 Phe Pro Gly Cys Ser Phe Ser Ile Phe
 1 5

<210> 291
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 168 (peptide 1145.25)

<400> 291
 Leu Pro Gly Cys Met Phe Ser Ile Phe
 1 5

<210> 292
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 169 (peptide 1292.24)

<400> 292
 Leu Pro Gly Cys Ser Phe Ser Ile Ile
 1 5

79iiii

<210> 293
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 168 (peptide 1145.14)

<400> 293
 Leu Pro Val Cys Ser Phe Ser Ile Phe
 1 5

<210> 294
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HCV Core 168 (peptide 1145.15)

<400> 294
 Leu Pro Gly Cys Ser Phe Ser Tyr Phe
 1 5

<210> 295
 <211> 26
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV1 GAG 294-319

<400> 295
 Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val
 1 5 10 15
 Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp
 20 25

<210> 296
 <211> 22
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV gag 298-319

<400> 296
 Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser
 1 5 10 15
 Pro Thr Ser Ile Leu Asp
 20

<210> 297
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>

79jjjj

<223> HIV1 GAG 298 (peptide 27.0313)

<400> 297

Lys	Arg	Trp	Ile	Ile	Leu	Gly	Leu	Asn	Lys	Ile	Val	Arg	Met	Tyr
1				5				10					15	

<210> 298

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 GAG 294 (peptide 27.0311)

<400> 298

Gly	Glu	Ile	Tyr	Lys	Arg	Trp	Ile	Ile	Leu	Gly	Leu	Asn	Lys	Ile
1				5				10					15	

<210> 299

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 POL 596 (peptide 27.0354)

<400> 299

Trp	Glu	Phe	Val	Asn	Thr	Pro	Pro	Leu	Val	Lys	Leu	Trp	Tyr	Gln
1				5				10					15	

<210> 300

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 POL 956 (peptide 27.0377)

<400> 300

Gln	Lys	Gln	Ile	Thr	Lys	Ile	Gln	Asn	Phe	Arg	Val	Tyr	Tyr	Arg
1				5				10					15	

<210> 301

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 POL 711-726

<400> 301

Glu	Lys	Val	Tyr	Leu	Ala	Trp	Val	Pro	Ala	His	Lys	Gly	Ile	Gly	Gly
1				5				10					15		

<210> 302

<211> 15

<212> PRT

<213> Artificial Sequence

79kkkk

<220>

<223> HIV POL 712 (peptide 1280.03)

<400> 302

Lys	Val	Tyr	Leu	Ala	Trp	Val	Pro	Ala	His	Lys	Gly	Ile	Gly	Gly
1				5					10				15	

<210> 303

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 POL 711 (peptide 27.0361)

<400> 303

Glu	Lys	Val	Tyr	Leu	Ala	Trp	Val	Pro	Ala	His	Lys	Gly	Ile	Gly
1				5					10				15	

<210> 304

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 gag 165-186

<400> 304

Pro	Ile	Val	Gln	Asn	Ile	Gln	Gly	Gln	Met	Val	His	Gln	Ala	Ile	Ser
1				5					10					15	
Pro	Arg	Thr	Leu	Asn	Ala										
			20												

<210> 305

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 GAG 171 (peptide 27.0304)

<400> 305

Gln	Gly	Gln	Met	Val	His	Gln	Ala	Ile	Ser	Pro	Arg	Thr	Leu	Asn
1				5					10				15	

<210> 306

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 ENV 729 (peptide 27.0297)

<400> 306

Gln	His	Leu	Leu	Gln	Leu	Thr	Val	Trp	Gly	Ile	Lys	Gln	Leu	Gln
1				5					10				15	

<210> 307

<211> 15

7911111

<212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV1 POL 335 (peptide 27.0344)

<400> 307
 Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro
 1 5 10 15

<210> 308
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV1 ENV 566 (peptide F091.15)

<400> 308
 Ile Lys Gln Phe Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr
 1 5 10 15

<210> 309
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV1 POL 303 (peptide 27.0341)

<400> 309
 Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu
 1 5 10 15

<210> 310
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV1 POL 758 (peptide 27.0364)

<400> 310
 His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 1 5 10 15

<210> 311
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HIV1 POL 915 (peptide 27.0373)

<400> 311
 Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg
 1 5 10 15

<210> 312
 <211> 22
 <212> PRT

79mmmm

<213> Artificial Sequence

<220>

<223> HIV GAG 245

<400> 312

Asp	Arg	Val	His	Pro	Val	His	Ala	Gly	Pro	Ile	Ala	Pro	Gly	Gln	Met
1				5					10					15	
Arg	Glu	Pro	Arg	Gly	Ser										
				20											

<210> 313

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV gag 195-220

<400> 313

Ala	Phe	Ser	Pro	Glu	Val	Ile	Pro	Met	Phe	Ser	Ala	Leu	Ser	Glu	Gly
1				5					10					15	
Ala	Thr	Pro	Gln	Asp	Leu	Asn	Thr	Met	Leu						
				20				25							

<210> 314

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV gag 195-216

<400> 314

Ala	Phe	Ser	Pro	Glu	Val	Ile	Pro	Met	Phe	Ser	Ala	Leu	Ser	Glu	Gly
1				5					10					15	
Ala	Thr	Pro	Gln	Asp	Leu										
				20											

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<220>

<223> HIV gag 205 (peptide 200.06)

<400> 315

Ser	Ala	Leu	Ser	Glu	Gly	Ala	Thr	Pro	Gln	Asp	Leu	Asn	Thr	Met	Leu
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<223> HIV gag 197 (peptide 27.0307)

<400> 316

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

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 <223> HIV gag 275

<400> 317
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 1 5 10 15
 Gly Glu Ile Tyr Lys Arg
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 <223> HIV gag 276 (peptide 27.0310)

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 Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val
 1 5 10 15

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<400> 319
 Tyr Arg Lys Ile Leu Arg Gln Arg Lys Ile Asp Arg Leu Ile Asp
 1 5 10 15

<210> 320
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<400> 320
 Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln
 1 5 10 15

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<220>

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<223> HIV POL 674 (peptide 35.0127)

<400> 321

Glu	Val	Asn	Ile	Val	Thr	Asp	Ser	Gln	Tyr	Ala	Leu	Gly	Ile	Ile
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<223> HIV POL 619 (peptide 35.0125)

<400> 322

Ala	Glu	Thr	Phe	Tyr	Val	Asp	Gly	Ala	Ala	Asn	Arg	Glu	Thr	Lys
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<211> 15

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<223> HIV POL 989 (peptide 35.0133)

<400> 323

Gly	Ala	Val	Val	Ile	Gln	Asp	Asn	Ser	Asp	Ile	Lys	Val	Val	Pro
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<220>

<223> HIV1 POL 70 (peptide 25.0148)

<400> 324

Met	Ala	Ser	Asp	Phe	Asn	Leu	Pro	Pro	Val
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<220>

<223> HIV gag 397 (peptide 1069.32)

<400> 325

Val	Leu	Ala	Glu	Ala	Met	Ser	Gln	Val
1				5				

<210> 326

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<220>

<223> HIV1 POL 87 (peptide 25.0062)

<400> 326

Lys Leu Val Gly Lys Leu Asn Trp Ala
 1 5

<210> 327

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> HIV1 NEF 62 (peptide 25.0039)

<400> 327

Leu Thr Phe Gly Trp Cys Phe Lys Leu
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<223> HIV1 GAG 34 (peptide 25. 0035)

<400> 328

Met Thr Asn Asn Pro Pro Ile Pro Val
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<223> HIV1 VPR 72 (peptide 25.0057)

<400> 329

Arg Ile Leu Gln Gln Leu Leu Phe Ile
 1 5

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<223> HIV POL 1434 (peptide 1.0944)

<400> 330

Ala Val Phe Ile His Asn Phe Lys Arg
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<220>

<223> HIV POL 1474 (peptide 1.1056)

<400> 331

Lys	Ile	Gln	Asn	Phe	Arg	Val	Tyr	Tyr	Arg
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<223> HIV pol 1432 (peptide 1069.49)

<400> 332

Gln	Met	Ala	Val	Phe	Ile	His	Asn	Phe	Lys
1				5				10	

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<220>

<223> HIV pol 337 (peptide 966.0102)

<400> 333

Ala	Ile	Phe	Gln	Ser	Ser	Met	Thr	Lys
1				5				

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<223> HIV pol 909 (peptide 1150.14)

<400> 334

Met	Ala	Val	Phe	Ile	His	Asn	Phe	Lys
1				5				

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<220>

<223> HIV nef 73-82 (peptide 940.03)

<400> 335

Gln	Val	Pro	Leu	Arg	Pro	Met	Thr	Tyr	Lys
1				5				10	

<210> 336

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<213> Artificial Sequence

<220>

<223> HIV1 ENV 81 (peptide 25.0175)

<400> 336

Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys
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<210> 337

<211> 10

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<220>

<223> HIV POL 65 (peptide 25.0209)

<400> 337

Val Thr Ile Lys Ile Gly Gly Gln Leu Lys
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<220>

<223> HIV nef 84-92 (peptide 1146.01)

<400> 338

Phe Pro Val Arg Pro Gln Val Pro Leu
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<210> 339

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<220>

<223> HIV env 293 (peptide 29.0060)

<400> 339

Ile Pro Ile His Tyr Cys Ala Pro Ala
1 5

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<223> HIV POL 171 (peptide 15.0073)

<400> 340

Phe Pro Ile Ser Pro Ile Glu Thr Val
1 5

<210> 341

<211> 9

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<212> PRT
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Cys Pro Lys Val Ser Phe Glu Pro Ile
1          5

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<223> HIV pol 883 (peptide 29.0107)

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Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val
1          5          10

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<223> HIV1 POL 96 (peptide 25.0151)

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Cys Thr Leu Asn Phe Pro Ile Ser Pro Ile
1          5          10

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<220>
<223> HIV1 NEF 62 (peptide 25.0143)

<400> 344
Leu Thr Pro Gly Trp Cys Phe Lys Leu Val
1          5          10

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<220>
<223> HIV1 POL 83 (peptide 25.0043)

<400> 345
Tyr Thr Ala Phe Thr Ile Pro Ser Ile
1          5

<210> 346

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<211> 9
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 1 5

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 Leu Val Gly Pro Thr Pro Val Asn Ile
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<220>
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 Ser Leu Leu Asn Ala Thr Asp Ile Ala Val
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 Thr Leu Asn Phe Pro Ile Ser Pro Ile
 1 5

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 Ile Val Ile Trp Gly Lys Thr Pro Lys
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<210> 356
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<220>
 <223> HIV1 GAG 45 (peptide 25.0064)

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 1 5

<210> 357
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 1 5

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 1 5 10

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 1 5

<210> 365
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<220>

<223> HIV1 POL 65 (peptide 25.0095)

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Thr Ile Lys Ile Gly Gly Gln Leu Lys
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<210> 366

<211> 9

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<223> HIV1 ENV 82 (peptide 25.0078)

<400> 366

Thr Leu Phe Cys Ala Ser Asp Ala Lys
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<210> 367

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<223> HIV1 VIF 83 (peptide 25.0104)

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Val Met Ile Val Trp Gln Val Asp Arg
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<223> HIV env 48 (peptide 1069.47)

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Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys
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<223> HIV GAG 507 (peptide 15.0268)

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Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe
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<210> 370

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<220>

<223> HIV GAG 248 (peptide 1292.13)

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His Pro Val His Ala Gly Pro Ile Ala

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<210> 371

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Val Pro Leu Gln Leu Pro Pro Leu

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Glu Val Asn Ile Val Thr Asp Ser Gln Tyr

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Phe Arg Asp Tyr Val Asp Arg Phe Tyr

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<223> HIV1 ENV 69 (peptide 25.0113)

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Ile Trp Gly Cys Ser Gly Lys Leu Ile

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<213> Artificial Sequence

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<223> HIV1 VPR 92 (peptide 25.0127)

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Ile Tyr Glu Thr Tyr Gly Asp Thr Trp
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<210> 376

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<223> HIV pol 1036 (peptide 1069.60)

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Ile Tyr Gln Glu Pro Phe Lys Asn Leu
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<223> HIV pol 359 (peptide 2.0129)

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Ile Tyr Gln Tyr Met Asp Asp Leu Tyr
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Pro Tyr Asn Glu Trp Thr Leu Glu Leu
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<223> HIV1 POL 74 (peptide 25.0123)

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Pro Tyr Asn Thr Pro Val Phe Ala Ile
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Thr Tyr Gln Ile Tyr Gln Glu Pro Phe
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<220>
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Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr
1          5          10

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<210> 385

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<211> 9
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<220>
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<210> 386
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<220>
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<400> 386
 Val Trp Lys Glu Ala Thr Thr Thr Leu Phe
 1 5 10

<210> 387
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 Tyr Met Gln Ala Thr Trp Ile Pro Glu Trp
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 Ser Leu Leu Asn Ala Thr Ala Ile Ala Val
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<210> 389
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<220>
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<220>
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1 5

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 1 5

<210> 401
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<220>
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<400> 402
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<400> 403
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 1 5

<210> 404
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<220>

<223> HIV GAG 248 (peptide 1292.14)

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<210> 405

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<223> HIV POL 179 (peptide 1292.09)

<400> 405

Phe Pro Ile Ser Pro Ile Glu Thr Ile
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<210> 406

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<220>

<223> HIV nef 84-92 analog (peptide 1145.02)

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<210> 407

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<223> HIV nef 84-92 analog (peptide 1145.22)

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Phe Pro Val Arg Met Gln Val Pro Leu
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<210> 408

<211> 9

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<223> HIV nef 84-92(a) (peptide 1181.04)

<400> 408

Phe Pro Val Arg Pro Gln Val Pro Met
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<210> 409

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<220>

<223> HIV nef 84-92(a) (peptide 1181.01)

<400> 409

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<400> 410

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5

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Phe Pro Val Arg Pro Gln Val Pro Phe

1

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<223> HIV nef 84-92(a) (peptide 1181.06)

<400> 412

Phe Pro Val Arg Pro Gln Val Pro Trp

1

5

<210> 413

<211> 16

<212> PRT

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<223> Pf SSP2 61 (peptide F125.04)

<400> 413

Arg His Asn Trp Val Asn His Ala Val Pro Leu Ala Met Lys Leu Ile

1

5

10

15

<210> 414

<211> 15

<212> PRT

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<213> Artificial Sequence

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<223> Pf SSP2 62 (peptide 1188.34)

<400> 414

His	Asn	Trp	Val	Asn	His	Ala	Val	Pro	Leu	Ala	Met	Lys	Leu	Ile
1				5					10				15	

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<212> PRT

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<220>

<223> Pf EXP1 71 (peptide 1188.16)

<400> 415

Lys	Ser	Lys	Tyr	Lys	Leu	Ala	Thr	Ser	Val	Leu	Ala	Gly	Leu	Leu
1				5					10				15	

<210> 416

<211> 18

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<220>

<223> Pf LSA1 13

<400> 416

Leu	Val	Asn	Leu	Leu	Ile	Phe	His	Ile	Asn	Gly	Lys	Ile	Ile	Lys	Asn
1				5					10					15	

Ser Glu

<210> 417

<211> 17

<212> PRT

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Ser

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<211> 15

<212> PRT

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79iiiiii

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 1 5 10 15

 <210> 419
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 <220>
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 1 5 10 15

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 1 5 10 15

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<400> 425

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<210> 428

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<210> 461
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THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding two or more heterologous peptide epitopes, wherein the heterologous peptide epitopes comprise two class II restricted helper T lymphocyte (HTL) hepatitis B virus (HBV) epitopes or a class I restricted cytotoxic T lymphocyte (CTL) HBV peptide epitope and a universal class II restricted HTL peptide epitope,

wherein at least one of said class II restricted HTL HBV peptide epitopes is selected from the group consisting of: KQAFTFSPTYKAFLC; LQSLTNLLSSNLSWL; AGFFLLTRILTIPQS; GTSFVYVPSALNPAD; GVVWIRTPPAYRPPNA; SFGVWIRTPPAYRPP; RHYLHTLWKAGILYK; PFLLAQFTSAICSVV; LVPFVQWFVGLSPTV; LHLYSHPIILGFRKI; KQCFRKLVPNRPIDW; AANWILRGTSFVYVP; LCQVFADATPTGWGL; ESRLVVDFSQFSRGN; VGPLTVNEKRRLKLI; and NLSWLSLDVSAAAFYH

and further wherein said class I restricted CTL HBV peptide epitope is selected from the group consisting of: WLSLLVPFV; GLSRYVARL; HTLWKAGILYK; STLPETTVVRR; LPSDFFPSV; TPARVTGGVF; LLVPFVQWFV; FLLAQFTSAI; VLLDYQGMLPV; LVPFVQWFV; LLAQFTSAI; NLSWLSIDV; LLSSNLSWL; KLHLYSHPI; FLLAQFTSA; GLLGWSPQA; HLYSHPHL; PLLPIFFCL; VLQAGFFLL; YMDDVVLGA; RLVVDFSQFSR; GVVWIRTPPAYR; SSAGPCALR; KVFVLGGCR; CALRFTSAR VSFGVWIR; TLPETTVVRRR; SVVRRAPPH; FVLGGCRHK; FTFSPITYK; AFTESPTYK; FPHCLAFSYM; YPALMPLYA; LPVCAFSSA; HPAAMPHELL; YPALMPLYACI; YPALMPLY; FPHCLAFSY; AYRPPNAPI; DLLDTASALY; EYLVSFVGVWI; FAAPFTQCGY; GYPALMPLY; HTLWKAGILY; ILLCLIFLL; KVGNTFTGLY; KYTSFPWLL; LLDTSALY; LSLDVSAAFY; LYSHPHLGF; MMWYWGPSLY; MSTTDLEAY; PLDKGIKPY; PTTGRTSLY; PWTHKVGNF; RWMCLRRFI; RWMCLRRFH; SFCGSPYSW; SLDVSAAAFY; SWLSLLVPF;

SWPKFAVPNL; SWWTSLNFL; SYQHFRKLLL; WFHISCLTF; WLWGMDIDPY;
 WMMWYWGPSLY; FLPSDFFPSI; YMDDVVLGV; FLPSDYFPSV; STLPETYVVR;R;
 FPIPSSWAF; FPHCLAFSL; FPHCLAFAL; IPITSSWAF; IIPMSWAF; IPILSSWAF;
 FPVCLAFSY and FPHCLAFAY.

2. The expression vector of claim 1, wherein the heterologous peptide epitopes comprise two or more heterologous class II restricted HTL HBV peptide epitopes.

3. The expression vector of claim 1, wherein the heterologous peptide epitopes comprise a class I restricted CTL HBV peptide epitope and a universal class II restricted HTL peptide epitope.

4. The expression vector of claim 3, wherein the heterologous peptide epitopes comprise two or more class II restricted HTL HBV peptide epitopes.

5. The expression vector of claim 2, wherein the heterologous peptide epitopes further comprise one or more class I restricted CTL HBV peptide epitopes.

6. The expression vector of claim 3, 4 or 5, wherein the class I restricted CTL peptide epitope comprises a structural motif for an HLA supertype, whereby the peptide class I restricted CTL epitope binds to two or more members of the supertype with an affinity of greater than 500 nM.

7. The expression vector of claim 3 or 5, wherein the heterologous peptide epitopes comprise two or more class I restricted CTL HBV peptide epitopes.

8. The expression vector of claim 4, 5 or 7, wherein the class I restricted CTL peptide epitopes have structural motifs that provide binding affinity for more than one HLA allele supertype.

9. The expression vector of claim 2 or 5, wherein the heterologous peptide epitopes further comprise a universal class II restricted HTL epitope.
10. The expression vector of claim 1, 3, 4, or 9, wherein the universal class II restricted HTL epitope is a pan DR epitope.
11. The expression vector of claim 10, wherein the pan DR epitope has the sequence AlaLysPheValAlaAlaTrpThrLeuLysAlaAlaAla (SEQ ID NO:52).
12. The expression vector of any one of claims 1 to 10, wherein the vector comprises pMin.1 (SEQ ID NO:38) or pEP2 (SEQ ID NO:35).
13. The expression vector of any one of claims 1 to 12, wherein the MHC targeting sequence comprises a signal sequence of a polypeptide selected from the group consisting of the Ii protein, LAMP-I, HLS-DM, HLA-DO, H2-DO, influenza matrix protein, hepatitis B surface antigen, hepatitis B virus core antigen, Ty particle, Ig- α protein, Ig- β protein, and Ig kappa chain.
14. The expression vector of any one of claims 1 to 13, wherein the expression vector further comprises a second promoter sequence operably linked to a third nucleotide sequence encoding one or more heterologous class II restricted HTL or class I restricted CTL peptide epitopes.
15. Use of the expression vector of any one of claims 1 to 14 for inducing an immune response *in vivo* in a mammalian subject.
16. Use of the expression vector of any one of claims 1 to 14 for preparation of a medicament for inducing an immune response *in vivo* in a mammalian subject.

17. A composition comprising a pharmaceutically acceptable carrier and the expression vector of any one of claims 1 to 14 for use in inducing an immune response *in vivo* in a mammalian subject.

18. A composition comprising a pharmaceutically acceptable carrier and an expression vector for use in inducing an immune response *in vivo* in a mammalian subject, wherein the expression vector comprises a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding one or more heterologous human class II restricted helper T lymphocyte (HTL) hepatitis B virus (HBV) peptide epitopes,

wherein at least one of said one or more HBV peptide class II restricted HTL HBV peptide epitopes is selected from the group consisting of: KQAFTFSPTYKAFLC; LQSLTNLLSSNLSWL; AGFFLLTRILTIPQS; GTSFVYVPSALNPAD; GVVWIRTPPAYRPPNA; SFGVWIRTPPAYRPP; RHYLHTLWKAGILYK; PFLLAQFTSAICSVV; LVPFVQWFVGLSPTV; LHLYSHPIILGFRKI; KQCFRKLVPVNRPIDW; AANWILRGTSFVYVP; LCQVFADATPTGWGL; ESRLVVDFSQFSRGN; VGPLTVNEKRRLKLI; and NLSWLSLDVSAAFYH.

19. The composition of claim 18, wherein the second nucleotide sequence encodes two or more heterologous class II restricted HTL HBV peptide epitopes.

20. The composition of claim 18 or 19, wherein the second nucleotide sequence further encodes one or more heterologous class I restricted CTL HBV peptide epitopes.

21. The composition of claim 20, wherein the class I restricted CTL peptide epitope comprises a structural motif for an HLA supertype, whereby the peptide epitope binds to two or more members of the supertype with an affinity of greater than 500 nM.

22. The composition of claim 20, wherein the class I restricted CTL peptide epitopes have structural motifs that provide binding affinity for more than one HLA allele supertype.

23. The composition of any one of claims 18 to 22, wherein the second nucleotide sequence further encodes a universal class II restricted HTL peptide epitope.

24. The composition of claim 23, wherein the universal class II restricted HTL epitope is a pan DR epitope.

25. The composition of claim 24, wherein the pan DR epitope has the sequence AlaLysPheValAlaAlaTrpThrLeuLysAlaAlaAla (SEQ ID NO:52).

26. The composition of any one of claims 18 to 25, wherein the MHC targeting sequence comprises a signal sequence of a polypeptide selected from the group consisting of Ii protein, LAMP-I, HLS-DM, HLA-DO, H2-DO, influenza matrix protein, hepatitis B surface antigen, hepatitis B virus core antigen, Ty particle, Ig- α protein, Ig- β protein, and Ig kappa chain.

27. The composition of any one of claims 18 to 26, wherein the expression vector further comprises a second promoter sequence operably linked to a third nucleotide sequence encoding one or more heterologous class II restricted HTL or class I restricted CTL peptide epitopes.

28. Use of an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding one or more heterologous human class II restricted helper T lymphocyte (HTL) hepatitis B virus (HBV) peptide epitopes for inducing an immune response *in vivo* in a mammalian subject,

wherein at least one of said one or more HBV peptide class II restricted HTL HBV peptide epitopes is selected from the group consisting of: KQAF^TFSPTYKAFLC; LQSLTNLLSSNLSWL; AGFFLLTRILTIPQS; GTSFVYVPSALNPAD; GVVIRTPPAYRPPNA; SFGVWIRTPPAYRPP; RHYLHTLWKAGILYK;

PFLLAQFTSAICSVV; LVPFVQWVGLSPTV; LHLYSHPIILGFRKI;
 KQCFRKLPVNRPIDW; AANWILRGTSFVYVP;
 LCQVFADATPTGWGL; ESRLVVDFSQFSRGN; VGPLTVNEKRRLKLI; and
 NLSWLSLDVSAAFYH.

29. Use of an expression vector comprising a promoter operably linked to a first nucleotide sequence encoding a major histocompatibility (MHC) targeting sequence fused to a second nucleotide sequence encoding one or more heterologous human class II restricted helper T lymphocyte (HTL) hepatitis B virus (HBV) peptide epitopes for preparation of a medicament for inducing an immune response *in vivo* in a mammalian subject,

wherein at least one of said HBV peptide class II restricted HTL HBV peptide epitopes is selected from the group consisting of: KQAFSTFSPTYKAFLC;
 LQSLTNLLSSNLSWL; AGFFLLTRILTIPQS; GTSFVYVPSALNPAD;
 GVWIRTPPAYRPPNA; SFGVWIRTPPAYRPP; RHYLHTLWKAGILYK;
 PFLLAQFTSAICSVV; LVPFVQWVGLSPTV; LHLYSHPIILGFRKI;
 KQCFRKLPVNRPIDW; AANWILRGTSFVYVP; LCQVFADATPTGWGL;
 ESRLVVDFSQFSRGN; VGPLTVNEKRRLKLI; and NLSWLSLDVSAAFYH.

30. The use of claim 28 or 29, wherein the second nucleotide sequence encodes two or more heterologous class II restricted HTL HBV peptide epitopes.

31. The use of claim 28, 29 or 30, wherein the second nucleotide sequence further encodes one or more heterologous class I restricted CTL HBV peptide epitopes.

32. The use of claim 31, wherein the class I restricted CTL peptide epitope comprises a structural motif for an HLA supertype, whereby the peptide epitope binds to two or more members of the supertype with an affinity of greater than 500 nM.

33. The use of claim 31, wherein the class I restricted CTL peptide epitopes have structural motifs that provide binding affinity for more than one HLA allele supertype.
34. The use of any one of claims 28 to 33, wherein the second nucleotide sequence further encodes a universal class II restricted HTL peptide epitope.
35. The use of claim 34, wherein the universal class II restricted HTL epitope is a pan DR epitope.
36. The use of claim 35, wherein the pan DR epitope has the sequence AlaLysPheValAlaAlaTrpThrLeuLysAlaAlaAla (SEQ ID NO:52).
37. The use of any one of claims 28 to 36, wherein the MHC targeting sequence comprises a signal sequence of a polypeptide selected from the group consisting of the Ii protein, LAMP-I, HLS-DM, HLA-DO, H2-DO, influenza matrix protein, hepatitis B surface antigen, hepatitis B virus core antigen, Ty particle, Ig- α protein, Ig- β protein, and Ig kappa chain.
38. The use of any one of claims 28 to 37, wherein the expression vector further comprises a second promoter sequence operably linked to a third nucleotide sequence encoding one or more heterologous class II restricted HTL or class I restricted CTL peptide epitopes.

```

      10      20      30      40      50      60      70
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CGATCGCGCGCGTGGTACCTACTGGTTGCGCTGGAGTAGAGATTGGTACTCGTTAACGGGTATGACCCGT
      M D D Q R D L I S N H E Q L P I L G>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
ACCGCCCTAGAGAGCCAGAAAGGTGCAGCCGTGGAGCTCTGTACACCGGTGTTTCTGTCCCTGGTGGCTCT
TGGCGGGATCTCTCGGTCTTTCCACGTCCGCACCTCGAGACATGTGGCCACAAAGACAGGACCCCGAGA
N R P R E P E R C S R G A L Y T G V S V L V A L>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
GCTCTTGGCTGGGCGAGGCCACCACTGCTTACTTCTGTACCAGCAACAGGGCCGCCCTAGACAAGCTGACC
CGAGAACCGACCCGTCCGCTGGTGAAGAATGAAGGACATGGTCTGTCCCGCGGATCTGTTCGACTGG
      L L A G Q A T T A Y F L Y Q Q G R L D K L T>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
ATCACCTCCAGAACCTGCACTGGAGAGCCTTCGCATGAAGCTTCCGAAATCTGCCAAACCTGTGGCCA
TAGTGGAGGCTCTTGGACGTGACCTCTCGGAAGCGTACTTCGAAGGCTTTAGACGGTTTGGACACCGGT
      I T S Q N L Q L E S L R M K L P K S A K P V A>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
AGTTCTGTGGCTGCCTGGACCCCTGAAGGCTGCCGCTATGTCCATGGATAACATGCTCCTTGGGCCTGTGAA
TCAAGCACCGACCGACCTGGGACTTCCGACGGCGATACAGGTACCTATTGTACGAGGAACCCGGACACTT
      K F V A A W T L K A A A M S M D N M L L G P V K>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
GAACGTTACCAAGTACGSCAACATGACCCAGGACCATGTGATGCATCTGCTCACGAGGTCTGGACCCCTG
CTTGCAATGGTTTCATGCCGTTGTACTGGGTCTGGTACACTACGTAGACGAGTGCTCCAGACCTGGGGAC
      N V T K Y G N N T Q D H V M H L L T R S G P L>

      430      440      450      460      470      480      490
      *      *      *      *      *      *      *
GAGTACCCCGAGCTGAAGGGGACCTTCCAGAGAATCTGAAGCATCTTAAGAACTCCATGGATGGCGTGA
CTCATGGGCGTCCACTTCCCTGGGAAGGCTCTCTTAGACTTCGTAGAATTCTTGAGGTACCTACCGCACT
      E Y P Q L K G T F P E N L K H L K N S M D G V>

      500      510      520      530      540      550      560
      *      *      *      *      *      *      *
ACTGGAAGATCTTCGAGAGCTGGATGAAGCAGTGGCTCTTGTGTTGAGATGAGCAAGAACTCCCTGGAGGA
TGACCTTCTAGAAGCTCTCGACCTACTTCGTACCGAGAACAACCTCTACTCGTTCTTGAGGGACCTCCT
      N W K I F E S W M K Q W L L F E M S K N S L E E>

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *
GAAGAAGCCCCACCGAGGCTCCACCTAAAGAGCCACTGGACATGGAAGACCTATCTTCTGGCCTGGGAGTG
CTTCTTCGGGTGGCTCCGAGGTGGATTCTCGGTGACCTGTACCTTCTGGATAGAAGACCGGACCTCAC
      K K P T E A P P K E P L D M E D L S S G L G V>

      640      650      660
      *      *      *
ACCAGGCAGGAACTGGGTCAAGTCACCCCTGTGAGGTACC
TGGTCCGTCTTGAACCACTTCAGTGGGACACTCCATGG
      T R Q E L G Q V T L *>

```

FIGURE 1

```

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      *      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGATGACCAACGCGACCTCATCTCTAACCATGAGCAATTGCCCATACTGGGCA
CGATCGCGGCGGTGGTACCTACTGGTTGCGCTGGAGTAGAGATTGGTACTCGTTAACGGGTATGACCCGT
      M D D Q R D L I S N H E Q L P I L G>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
ACCGCCCTAGAGAGCCAGAAAGGTGCAGCCGTGGAGCTCTGTACACCGGTGTTTCTGTCTGGTGGCTCT
TGGCGGGATCTCTCGGTCTTTCCACGTCGGCACCTCGAGACATGTGGCCCAAAGACAGGACCACCGAGA
N R P R E P E R C S R G A L Y T G V S V L V A L>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
GCTCTTGGCTGGGCAGGCCACCACTGCTTACTTCCTGTACCAGCAACAGGGCCGCCTAGACAAGCTGACC
CGAGAACCGACCCGTCCGGTGGTGACGAATGAAGGACATGGTCGTTGTCCCGGCGGATCTGTTTCGACTGG
      L L A G Q A T T A Y F L Y Q Q Q G R L D K L T>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
ATCACCTCCCAGAACCTGCAACTGGAGAGCCTTCGCATGAAGCTTATCAGCCAGGCTGTGCACGCCGCTC
TAGTGGAGGGTCTTGGACSTTGACCTCTCGGAAGCGTACTTCGAATAGTCGGTCCGACACGTGCGGCGAG
      I T S Q N L Q L E S L R M K L I S Q A V H A A>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
ACGCCGAAATCAACGAAGCTGGAAGAACCCCTCCAGCTTATCGCCCTCCAAACGCTCCTATCCTGTTCTT
TGCGGCTTTAGTTGCTTCGACCTTCTTGGGGAGGTGGAATAGCGGGAGGTTTGCAGGATAGGACAAGAA
H A E I N E A G R T P P A Y R P P N A P I L F F>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
TCTGCTGACCAGAATCCTGACAATCCCCCAGTCCCTGGACGCCAAGTTTCGTGGCTGCCTGGACCCTGAAG
AGACGACTGGTCTTAGGACTGTTAGGGGGTCAGGGACCTGCGGTTCAAGCACCGACGGACCTGGGACTTC
      L L T R I L T I P Q S L D A K F V A A W T L K>

      430
      *      *      *
GCTGCCGCTTGAGGTACC
CGACGGCGAACTCCATGG
      A A A *>

```

FIGURE 2

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGATGACCAACGCGACCTCATCTCTAACCATGAGCAATTGCCCATACTGGGCA
CGATCGCGCGCGGTGGTACCTACTGGTTGCGCTGGAGTAGAGATTGGTACTCGTTAACGGGTATGACCCGT
      M D D Q R D L I S N H E Q L P I L G>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
ACCGCCCTAGAGAGCCAGAAAGGTGCAGCCGTGGAGCTCTGTACACCGGTGTTTCTGTCTTGGTGGCTCT
TGGCGGGATCTCTCGGTCTTTCCACGTCCGCACCTCGAGACATGTGGCCACAAAGACAGGACCACCGAGA
N R P R E P E R C S R G A L Y T G V S V L V A L>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
GCTCTTGGCTGGGCAGGCCACCACTGCTTACTTCTGTACCAGCAACAGGGCCGCTAGACAAGCTGACC
CGAGAACCGACCCGTCCGGTGGTGACGAATGAAGGACATGGTTCGTTGCCCGCGGATCTGTTCCGACTGG
L L A G Q A T T A Y F L Y Q Q Q G R L D K L T>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
ATCACCTCCCAGAACCTGCAACTGGAGAGCCTTCGCATGAAGCTTATCAGCCAGGCTGTGCACGCEGCTC
TAGTGGAGGGTCTTGGACGTTGACCTCTCGGAAGCGTACTTCGAATAGTCCGTCCGACACGTGCGGCGAG
I T S Q N L Q L E S L R M K L I S Q A V R A A>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
ACGCCGAAATCAACGAAGCTGGAAGAACCCCTCCAGCTTATCGCCCTCCAAACGCTCCTATCCTGTTCTT
TGCGGCTTTAGTTGCTTCGACCTTCTTGGGGAGGTGCAATAGCGGGAGGTTTGCGAGGATAGGACAAGAA
H A E I N E A G R T P P A Y R P P N A P I L F F>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
TCTGCTGACCAGAATCCTGACAATCCCCAGTCCCTGGACGCCAAGTTCGTGGCTGCCTGGACCCTGAAG
AGACGACTGGTCTTAGGACTGTTAGGGGGTTCAGGGACCTGCGGTTCAAGCACCGACGGACCTGGGACTTC
L L T R I L T I P Q S L D A K F V A A W T L K>

      430      440      450      460      470      480      490
      *      *      *      *      *      *      *
GCTGCCGCTATGTCCATGGATAACATGCTCCTTGGGCCTGTGAAGAACGTTACCAAGTACGGCAACATGA
CGACGGCGATACAGGTACCTATTGTACGAGGAACCCGGACACTTCTTGCAATGGTTTCATGCCGTTGTACT
A A A M S M D N M L L G P V K N V T K Y G N M>

      500      510      520      530      540      550      560
      *      *      *      *      *      *      *
CCCAGGACCATGTGATGCATCTGCTCACGAGGTCTGGACCCCTGGAGTACCCGCAGCTGAAGGGGACCTT
GGGTCCTGGTACACTACGTAGACGAGTGCTCCAGACCTGGGGACCTCATGGGCGTCGACTTCCCCTGGAA
T Q D H V M H L L T R S G P L E Y P Q L K G T F>

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *
CCCAGAGAATCTGAAGCATCTTAAGAAGTCCATGGATGGCGTGAAGTGAAGATCTTCGAGAGCTGGATG
GGGTCTCTTAGACTTCGTAGAATTCTTGAGGTACCTACCGCACTTGACCTTCTAGAAGCTCTCGACCTAC
P E N L K H L K N S M D G V N W K I F E S W M>

```

FIGURE 3

```
      640      650      660      670      680      690      700
      *      *      *      *      *      *      *      *
AAGCAGTGGCTCTTGTGTTGAGATGAGCAAGAACTCCCTGGAGGAGAAGAAGCCCACCGAGGCTCCACCTA
TTCGTCACCGAGAACAACTCTACTCGTTCTTGAGGGACCTCCTCTTCTTCGGGTGGCTCCGAGGTGGAT
K Q W L L F E M S K N S L E E K K P T E A P P>

      710      720      730      740      750      760      770
      *      *      *      *      *      *      *      *
AAGAGCCACTGGACATGGAAGACCTATCTTCTGGCCTGGGAGTGACCAGGCAGGAACTGGGTCAAGTCAC
TTCTCGGTGACCTGTACCTTCTGGATAGAAGACCGGACCCTCACTGGTCCGTCCTTGACCCAGTTCAGTG
K E P L D M E D L S S G L G V T R Q E L G Q V T>

      780
      *      *
CCTGTGAGGTACC
GGACACTCCATGG
L *>
```

FIGURE 3 CONTINUED

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGGAATGCAGGTGCAGATCCAGAGCCTGTTTCTGCTCCTCCTGTGGGTGCCCCG
CGATCGCGGCGGTGGTACCCTTACGTCCACGTCTAGGTCTCGGACAAAGACGAGGAGGACACCCACGGGC
      M G M Q V Q I Q S L F L L L L W V P>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
GGTCCAGAGGAATCAGCCAGGCTGTGCACGCCGCTCAGCGCGAAATCAACGAAGCTGGAAGAACCCCTCC
CCAGGTCTCCTTAGTCGGTCCGACACGTGCGGCGAGTGGCGCTTTAGTTGCTTCGACCTTCTTGGGGAGG
G S R G I S Q A V H A A H A E I N E A G R T P P>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
AGCTTATCGCCCTCCAAACGCTCCTATCCTGTTCTTTCTGCTGACCAGAATCCTGACAATCCCCCAGTCC
TCGAATAGCGGGAGGTTTGGGAGGATAGGACAAGAAAGACGACTGGTCTTAGGACTGTTAGGGGGTCAGG
A Y R P P N A P I L F F L L T R I L T I P Q S>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
CTGGACGCCAAGTTCTGTGGCTGCCTGGACCCTGAAGGCTGCCGCTAACAACATGTTGATCCCCATTGCTG
GACCTGCGGTTCAAGCACCGACGGACCTGGGACTTCCGACGGCGATTGTTGTACAACACTAGGGGTAACGAC
L D A K F V A A W T L K A A A N N M L I P I A>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
TGGGCGGTGCCCTGGCAGGGCTGGTCTCATCGTCCTCATTGCCTACCTCATTGGCAGGAAGAGGAGTCA
ACCCGCCACGGGACCGTCCCGACCAGGAGTAGCAGGAGTAACGGATGGAGTAACCGTCCTTCTCCTCAGT
V G G A L A G L V L I V L I A Y L I G R K R S H>

      360      370
      *      *      *      *      *
CGCCGGCTATCAGACCATCTAGGGTACC
GCGGCCGATAGTCTGGTAGATCCCATGG
A G Y Q T I *>

```

FIGURE 4

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCGCCACCATGGCTGCACTCTGGCTGCTGCTGGTCCTCAGTCTGCACTGTATGGGGATCA
CGATCGCGGCGGTGGTACCGACGTGAGACCGACGACGACGACGAGGAGTCAGACGTGACATACCCCTAGT
      M A A L W L L L L V L S L H C M G I>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
GCCAGGCTGTGCACGCCGCTCACGCCGAAATCAACGAAGCTGGAAGAACCCCTCCAGCTTATCGCCCTCC
CGGTCCGACACGTGCGGCGAGTGCGGCTTTAGTTGCTTCGACCTTCTTGGGGAGGTGGAATAGCGGGAGG
S Q A V H A A H A E I N E A G R T P P A Y R P P>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
AAACGCTCCTATCCTGTTCTTTCTGCTGACCAGAATCCTGACAATCCCCCAGTCCCTGGACGCCAAGTTC
TTTGCGAGGATAGGACAAGAAAGACGACTGGTCTTAGGACTGTTAGGGGGTCAGGGACCTGCGGTTCAAG
N A P I L F F L L T R I L T I P Q S L D A K F>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
GTGGCTGCCTGGACCCTGAAGGCTGCCGCTAAGGTCTCTGTGTCTGCAGCCACCCTGGGCCTGGGCTTCA
CACCGACGGACCTGGGACTTCCGACGGCGATTCCAGAGACACAGACGTCGGTGGGACCCGGACCCGAAGT
V A A W T L K A A A K V S V S A A T L G L G F>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
TCATCTTCTGTGTTGGCTTCTTCAGATGGCGCAAGTCTCATTCCCTCCAGCTACACTCCTCTCCCTGGATC
AGTAGAAGACACAACCGAAGAAGTCTACCGCGTTTCAGAGTAAGGAGGTGATGTGAGGAGAGGGACCTAG
I I F C V G F F R W R K S H S S S Y T P L P G S>

      360      370      380
      *      *      *
CACCTACCCAGAAGGACGGCATTAGGGTACC
GTGGATGGGTCTTCCTGCCGTAATCCCATGG
T Y P E G R H *>

```

FIGURE 5

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGGCGCTGGGAGGGCCCCCTGGGTGGTGGCTCTGTTGGTGAACCTCATGAGGC
CGATCGCGGCGGTGGTACCCGCGACCCCTCCCGGGGGACCCACCACCGAGACAACCACTTGGAGTACTCCG
      M G A G R A P W V V A L L V N L M R>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
TGGATTCCATCAGCCAGGCTGTGCACGCCGCTCAGCGCGAATCAACGAAGCTGGAAGAACCCCTCCAGC
ACCTAAGGTAGTCGGTCCGACACGTGCGGCGAGTGCGGCTTTAGTTGCTTCGACCTTCTTGGGGAGGTCC
L D S I S Q A V H A A H A E I N E A G R T P P A>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
TTATCGCCCTCCAAACGCTCCTATCCTGTTCTTTCTGCTGACCAGAATCCTGACAATCCCCCAGTCCCTG
AATAGCGGGAGGTTTGGCAGGATAGGACAAGAAAGACGACTGGTCTTAGGACTGTTAGGGGGTCAGGGAC
Y R P P N A P I L F F L L T R I L T I P Q S L>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
GACGCCAAGTTCGTGGCTCCTGGACCCTGAAGGCTGCCGCTATACTGAGTGGAGCTGCAGTGTTCCTGC
CTGCGGTTCAAGCACCGACCGACCTGGGACTTCCGACGGCGATATGACTCACCTCGACGTCACAAGGACG
D A K F V A A W T L K A A A I L S G A A V F L>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
TTGGGCTGATTGTCTTCCTGGTGGGGGTTGTTATCCATCTCAAGGCTCAGAAAGCATCTGTGGAGACTCA
AACCCGACTAACAGAAGGACCACCCCAACAATAGGTAGAGTTCCGAGTCTTTCGTAGACACCTCTGAGT
L G L I V F L V G V V I H L K A Q K A S V E T Q>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
GCCTGGCAATGAGAGTAGGTCCCGGATGATGGAGCGGCTAACCAAGTTCAAGGCTGGACCGGGACATGTC
CGGACCGTTACTCTCATCCAGGGCCTACTACCTCGCCGATTGGTTCAAGTTCCGACCTGGCCCTGTACAG
P G N E S R S R M M E R L T K F K A G P G H V>

      430
      *      *
ACATGAGGTACC
TGTA TCCATGG
T *>

```

FIGURE 6

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCGCGCCACCATGGCCAAAGTTCTGGCTGCCTGGACCTGAAGGCTGCCGCTATGAGTCTTCTAA
CGATCGCGCGGGTGGTACCGGTTCAAGCACCGACGGACCTGGGACTTCCGACGGCGATACTCAGAAGATT
      M A E F V A A W T L K A A A M S L L>

      80      90      100     110     120     130     140
      *      *      *      *      *      *      *
CCGAGGTGGAACGTACGTTCTCTCTATCATCCCATCAGGCCCCCTCAAAGCCGAGATCGCGCAGAGACT
GGCTCCAGCTTTGCATGCAAGAGAGATAGTAGGGTAGTCCGGGGGAGTTTCCGGCTCTAGCGCGTCTCTGA
T E V E T Y V L S I I P S G P L K A E I A Q R L>

      150     160     170     180     190     200     210
      *      *      *      *      *      *      *
TGAGGATGTTTTGCGAGGGAAGAACACAGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAATC
ACTCCTACAAAACGTCCTCTTCTGTCTAGAACTCCGAGAGTACCTTACCGATTCTGTCTCTGGTTAG
E D V F A G K N T D L E A L M E W L K T R P I>

      220     230     240     250     260     270     280
      *      *      *      *      *      *      *
CTGTCACTCTGACTAAGGGAATTTTAGGGTTTGTGTTTACCGCTCACCGTGCCGAGTGAGCGAGGACTGC
GACAGTGGAGACTGATTCCCTTAAATCCCAAACACAAGTGGAGTGGCACGGGTCACTCGCTCCTGACG
L S P L T K G I L G F V F T L T V P S E R G L>

      290     300     310     320     330     340     350
      *      *      *      *      *      *      *
AGCGTAGACGATTTGTCCAAATGCCCTAAATGGGAATGGAGACCCAAACAACATGGACAGGGCAGTTAA
TCGCATCTGCTAAACAGGTTTACGGGATTTACCTTACCTCTGGGTTTGTGTACCTGTCCCGTCAATT
Q R R R F V Q N A L N G N G D P N N M D R A V K>

      360     370     380     390     400     410     420
      *      *      *      *      *      *      *
ACTATACAAGAAGCTGAAGAGGGAATGACATTCATGGAGCAAGGAAGTTGCACTCAGTTACTCAACT
TGATATGTTCTTCGACTTCTCCCTTACTGTAAGGTACCTCGTTTCTTCAACGTGAGTCAATGAGTTGA
L Y K K L K R E M T F H G A K E V A L S Y S T>

      430     440     450     460     470     480     490
      *      *      *      *      *      *      *
GGTGGCTTGGCAGTTGCATGGGTCTCATATACAACCGGATGGGAACAGTGACCACAGAAGTGGCTCTTG
CCACGGCAACGGTCAACGTACCCAGAGTATATGTTGGCCTACCTTGTCACTGGTGTCTTCAACGAGAAC
G A L A S C M E L I Y N R M G T V T T E V A L>

      500     510     520     530     540     550     560
      *      *      *      *      *      *      *
GCCTAGTATGTGCCACTTGTGAACAGATTGCTGATGCCCAACATCGGTCCACAGGCAGATGGCGACTAC
CGGATCATACAGGTGAACACTCTCTAACGACTACGGGTTGTAGCCAGGGTGTCCGTCTACCGCTGATG
G L V C A T C E Q I A D A Q H R S H R Q M A T T>

      570     580     590     600     610     620     630
      *      *      *      *      *      *      *
CACCAACCCACTAATCAGGCATGAGAACAGAATGGTACTAGCCAGCACTACGGCTAAGGCCATGGAGCAA
GTGGTTGGGTGATTAGTCCGTACTCTTGTCTTACCATGATCGGTCTGATGCCGATTCCGGTACCTCGTT
T N P L I R E E N R M V L A S T T A K A M E Q>

      640     650     660     670     680     690     700
      *      *      *      *      *      *      *
ATGGCTGGATCAAGTGAGCAGGCAAGCAGAGGCCATGGAAGTCGCAAGTCAGGCTAGACAAATGGTGCAGG
TACCGACCTAGTTCACTCGTCCGTCTCTCCGTACCTTACCGTTCACTCCGATCTGTTTACCACGTCC
M A G S S E Q A A E A M E V A S Q A R Q M V Q>

```

FIGURE 7

```
      710      720      730      740      750      760      770
      *      *      *      *      *      *      *
CAATGAGGACAATTGGGACTCACCTAGCTCCAGTGCAGGTCTAAAAGATGATCTTATTGAAAATTGCA
GTTACTCCTGTTAACCCCTGAGTGGGATCGAGGTCCAGATTTTCTACTAGAATAACTTTTAAACGT
A M R T I G T H P S S S A G L K D D L I E N L Q>

      780      790      800      810
      *      *      *      *      *      *
GGCTTACCAGAAACGGATGGGGGTGCAGATGCAGCGATTCAAGTGA
CCGAATGGTCTTTGCCCTACCCCCACGTCTACGTGCTAAGTTCACT
A Y Q K R M G V Q M Q R F K *>
```

FIGURE 7 CONTINUED

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGCCAAGTTGCTGGCTGCCTGGACCCTGAAGGCTGCCGCTCTCGAGATTGGGG
CGATCGCGGCGGTGGTACCGGTTCAAGCACCGACGGACCTGGGACTTCCGACGGCGAGAGCTCTAACCCC
      M A K F V A A W T L K A A A L E I G>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
GACCCCTGCCTGAACGCCGAGAACATCACATCAGGATTCCTAGGACCCCTTCTCGTGTTACAGGCGGGGTT
CTGGGACGGACTTGGCGCTCTTGTAGTGTAGTCCTAAGGATCCTGGGGAAGAGCACAATGTCCGCCCCAA
G P C L N A E N I T S G F L G P L L V L Q A G F>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
TTTCTTGTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTA
AAAGAACAACCTGTTCTTAGGAGTGTTATGGCGTCTCAGATCTGAGCACCACTGAAGAGAGTTAAAAGAT
F L L T R I L T I P Q S L D S W W T S L N F L>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
GGGGGAACCTACCGTGTGTCTTGGCCAAAATTGCGAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTG
CCCCCTTGATGGCACACAGAACCGGTTTAAAGCGTCAGGGSTTGGAGGTTAGTGAGTGGTGGAGAACAG
G G T T V C L G Q N S Q S P T S N H S P T S C>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
CTCCAACTTGTCTGTTATCGCTGGATGTGTCTGCGGCGTTTATCATCTTCTCTTCATCCTGCTGCT
GAGGTTGAACAGGACCAATAGCGACCTACACAGACGCCGCAAATAGTAGAAGGAGAAGTAGGACGACGA
P P T C P G Y R W M C L R R F I I F L F I L L L>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
ATGCCCTCATCTTCTTGTGGTTCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCTCTAATTCAGGA
TACGGAGTAGAAGAACAACCAAGAAGACCTGATAGTTCCATACAACGGGCAAACAGGAGATTAAAGTCTCT
C L I F L L V L L D Y Q G M L P V C P L I P G>

      430      440      450      460      470      480      490
      *      *      *      *      *      *      *
TCCTCAACAACAGCACGGGACCATGCCGGACCTGCATGACTACTGCTCAAGGAACCTCTATGTATCCCT
AGGAGTTGTTGGTCTGTCCTGGTACGGCCTGGACGTACTGATGACGAGTTCCTTGGAGATACATAGGGA
S S T T S T G P C R T C M T T A Q G T S M Y P>

      500      510      520      530      540      550      560
      *      *      *      *      *      *      *
CCTGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTTCGG
GGACAACGACATGGTTTGGAAAGCCTGCCTTTAACGTGGACATAAGGGTAGGGTAGTAGGACCCGAAAGCC
S C C C T K P S D G N C T C I P I P S S W A F G>

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *
AAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTTCAGTGG
TTTTAAGGATACCCCTACCCGGAGTCGGGCAAAGAGGACCGAGTCAAATGATCACGGTAAACAAGTCACC
K F L W E W A S A R F S W L S L L V P F V Q W>

```

FIGURE 8

```
      640      650      660      670      680      690      700
      *      *      *      *      *      *      *      *
TTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGT
AAGCATCCCCGAAAGGGGGTGACAAACCGAAAGTCAATATACCTACTACACCATAACCCCCGGTTCAGACA
F V G L S P T V W L S V I W M M W Y W G P S L>

      710      720      730      740      750      760      770
      *      *      *      *      *      *      *      *
ACAGCATCTTGAGTCCCTTTTACCGCTGTTACCAATTTCTTTTGTCTTTGGGTATACATTTAAACCCT
TGTCGTAGAACTCAGGGAAAAATGGCGACAATGGTTAAAAGAAAACAGAAACCCATATGTAAATTTGGGA
Y S I L S P F L P L L P I F F C L W V Y I *>

      780      790      800
      *      *      *      *      *      *
AACAAAACAAAGAGATGGGGTTACTCTCTAA
TTGTTTGTCTCTCTACCCCAATGAGAGATT
```

FIGURE 8 CONTINUED

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCGCGCCACCATGCCAGCGGGTCTAGAAGCCCTCAGAGCCCTGCCCTCTCCTCTCTTCTTCTCAT
CGATCGCGCGCGGTGGTACGGTCCCCCAGATCTTCGGGAGTCTCCGGACGGAGAGGAGGAGAAGAACAAGTA
      M P G G L E A L R A L P L L L F L S>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
ACGCCGTGTTTGGGTCCCCGATGCCAGGCCATCAGCCAGGCTGTGCACGCGCGCTCAGGCCCAAATCAACGA
TCCGGACAAACCCAGGGCCTACGGTCCGGTAGTCCGTCCGACACGTCCGGCGAGTCCGGCTTTAGTTGCT
Y A C L G P G C Q A I S Q A V H A A H A E I N E>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
AGCTCGAAGAACCCTCCAGCTTATCGCCCTCCAAACGCTCCTATCCTGTTCTTTCTGCTGACCAGAATC
TCGACCTTCTTGGGGAGGTGSAATAGCGGGAGGTTTCGAGGATAGGACAAGAAAGACGACTGCTCTTAG
      A G R T P P A Y R P P N A P I L F F L L T R I>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
CTGACAATCCCCCACTCCCTCCAGCCCAAGTTGGTGGCTGGCTGGACCCCTGAAGGCTGCCGCTGGCATCA
GACTGTTAGGGGGTCCAGGACCTCCGGTTCAAGCACCGACGGACCTGGGACTTCGGACGGCGACCCCTAGT
      L T I P Q S L D A K F V A A W T L K A A A G I>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
TCTTGGTGTTCCTGTCCAGTGGTGGCAGGGACCGTGGTGGTATTCAGGAAACGGTGGCAAAATGAGAAGTT
AGAACGACAAGACACGTCCAGCGGTCCCTGGCAGCAGGATAAGTCCCTTGGCACCGTTTACTCTTCAA
      I L L F C A V V P G T L L L F R K R W Q N E K F>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
TGGCGTGGACATGCCAGATCACTATGAAGATGAAAATCTCTATGAGGGCCCTGAACCTTCATGACTGTTCT
ACCCACCTGTACGGTCTACTGATACTTCTACTTTTAGAGATACTCCCCGACTTGGAACTACTGACAAGA
      G V D M P D D Y E D E N L Y E G L N L D D C S>

      430      440      450      460      470      480      490
      *      *      *      *      *      *      *
ATGTATGAGGACATCTCCAGGGGACTCCAGGGCACCTACCAGGATGTGGGCAACCTCCACATTGGAGATG
TACATACTCCTGTAGAGGTCCCTGAGGTCCCGTGGATGGTCCCTACACCCGTTGGAGGTGTAACCTCTAC
      M Y E D I S R G L Q G T Y Q D V G N L H I G D>

      500      510
      *      *      *
CCCAGCTGGAAAAGCCATGAGGTACC
GGGTGCACCTTTTCGGTACTCCATGG
      A Q L E K P *>

```

FIGURE 9

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCGCGCCACCATGCGCCACACTGGTGGTGTCTTCCATGCCCTGCCACTGGGTGTTGTTCCTGCTGC
CGATCGCGCGCGGTGGTACCGGTGTGACCACGACAGAAGGTACGGGACGGTGACCGACAACAAGGACGACC
      H A T L V L S S M P C H W L L F L L>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
TGCTCTTCTCAGGTGAGCGGATCAGCCAGGCTGTCCAGCGCGCTCAGCGCGAAATCAACGAAGCTGGAAG
ACGAGAAGAGTCCACTCGGCTAGTCCGTCCGACACGTGCGCGAGTGCGGCTTTAGTTGCTTCGACCTTC
L L F S G E P I S Q A V H A A H A E I N E A G R>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
AACCCCTCCAGCTTATCGCCCTCCAAACGCTCCTATCCTGTTCTTCTGCTGACCAGAATCCTGACAATC
TTGGGAGGTTGGAATAGCGGAGGTTTCCGAGGATAGGACAAGAAAGACGACTGGTCTTAGGACTGTTAG
T P P A Y R P P N A P I L F F L L T R I L T I>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
CCCCAGTCCCTGGACGCCAAGTTGCTGGCTGCGCTGACCGCTGAAGGCTGCCGCTATTATCTTGATCCAGA
GGGCTCAGCGACCTCGGTTCAAGCACCGACCGACCTGGGACTTCCGACCGCGATAATAGAACTAGGCTCT
P Q S L D A K F V A A W T L K A A A I I L I Q>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
CCCTCCTCATCATCCTCTTCATCATTTGTGCCCATCTTCTCTGCTACTTGACAAGGATGACGGCAAGGCTGG
GGGAGGAGTAGTAGGAGAAGTAGTAACACGGGTAGAAGGACGATGAACCTGTTCTTCTACTGCCGTTCCGACC
T L L I I L F I I V P I F L L L D K D D G K A G>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
GATCGAGGAAGATCACACCTATGAGGGCTTGAACATTGACCAGACAGCCACCTATGAAGACATAGTGA
CTACCTCCTTCTAGTGTGGATACTCCCGAAGTTGTAACCTGGTCTGTGCGGTGGATACTTCTGTATCACTGA
M E E D H T Y E G L N I D Q T A T Y E D I V T>

      430      440      450      460      470      480
      *      *      *      *      *      *
CTTCGGACAGGGGAGGTAAAGTGGTCCGTAGGAGAGCATCCAGGCCAGGAATGAGGTACC
GAAGCCTGTCCCTCCATTTCCACGCCATCCTCTCGTAGGTCCGGTCCCTTACTCCATGG
L R T G E V K W S V G E H P G Q E ">

```

FIGURE 10

```
      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCGCGCCACCATCGGAATGCAGGTGCAGATCCAGAGCCCTGTTTCTCCTCCTCTGTTGGTCCCCG
CGATCGCGCGCGGTGGTACCCCTTACGTCCACGTCTAGGTCTCGGACAAAGACGAGGAGGACACCCACCGGC
      M G M Q V Q I Q S L F L L L L W V P>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
GGTCCCGAGGAATCAGCCAGGCTGTGCACCCCGCTCAGCCCGAAATCAACGAAGCTCGAAGAACCCCTCC
CCAGGGCTCCTTAGTCCGTCCGACACGTGCCCGGAGTCCCGCTTTAGTTGCTTCGACCTTCTTGGCGAGG
G S R G I S Q A V H A A H A E I N E A G R T P P>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
AGCTTATCGCCCTCCAAACGCTCCTATCCTGTTCTTTCTGCTGACCAGAATCCTGACAATCCCCCAGTCC
TCGAATAGCGGGAGGTTTGGGAGGATAGGACAAGAAAGACGACTCGTCTTAGGACTGTTAGGGGGTCAGG
A Y R P P N A P I L F F L L T R I L T I P Q S>

      220      230      240      250      260
      *      *      *      *      *
CTGGACGCCAAAGTTCTGTGGCTCCCTGGACCCCTGAAGGCTGCCGCTTGAGGTACC
GACCTGCGGTTCAAGCACCGACCGACCTGGGACTTCCGACCGCGAACTCCATGG
L D A K F V A A W T L K A A A *>
```

FIGURE 11

TTCCCAG	ATG	CAC	AGG	AGG	AGA	AGC	AGG	AGC	TGT	CGG	GAA	GAT	CAG	AAG	49	
	Met	His	Arg	Arg	Arg	Ser	Arg	Ser	Cys	Arg	Glu	Asp	Gln	Lys		
	1				5				10							
CCA	GTC	ATG	GAT	GAC	CAG	CGC	GAC	CTT	ATC	TCC	AAC	AAT	GAG	CAA	CTG	97
Pro	Val	Met	Asp	Asp	Gln	Arg	Asp	Leu	Ile	Ser	Asn	Asn	Glu	Gln	Leu	
15					20				25						30	
CCC	ATG	CTG	GGC	CGG	CGC	CCT	GGG	GCC	CCG	GAG	AGC	AAG	TGC	AGC	CGC	145
Pro	Met	Leu	Gly	Arg	Arg	Pro	Gly	Ala	Pro	Glu	Ser	Lys	Cys	Ser	Arg	
				35				40					45			
GGA	GCC	CTG	TAC	ACA	GGC	TTT	TCC	ATC	CTG	GTG	ACT	CTG	CTC	CTC	GCT	193
Gly	Ala	Leu	Tyr	Thr	Gly	Phe	Ser	Ile	Leu	Val	Thr	Leu	Leu	Leu	Ala	
			50					55					60			
GGC	CAG	GCC	ACC	ACC	GCC	TAC	TTC	CTG	TAC	CAG	CAG	CAG	GGC	CGG	CTG	241
Gly	Gln	Ala	Thr	Thr	Ala	Tyr	Phe	Leu	Tyr	Gln	Gln	Gln	Gly	Arg	Leu	
		65					70					75				
GAC	AAA	CTG	ACA	GTC	ACC	TCC	CAG	AAC	CTG	CAG	CTG	GAG	AAC	CTG	CGC	289
Asp	Lys	Leu	Thr	Val	Thr	Ser	Gln	Asn	Leu	Gln	Leu	Glu	Asn	Leu	Arg	
	80						85					90				
ATG	AAG	CTT	CCC	AAG	CCT	CCC	AAG	CCT	GTG	AGC	AAG	ATG	CGC	ATG	GCC	337
Met	Lys	Leu	Pro	Lys	Pro	Pro	Lys	Pro	Val	Ser	Lys	Met	Arg	Met	Ala	
95					100					105					110	
ACC	CCG	CTG	CTG	ATG	CAG	GCG	CTG	CCC	ATG	GGA	GCC	CTG	CCC	CAG	GGG	385
Thr	Pro	Leu	Leu	Met	Gln	Ala	Leu	Pro	Met	Gly	Ala	Leu	Pro	Gln	Gly	
				115					120					125		
CCC	ATG	CAG	AAT	GCC	ACC	AAG	TAT	GGC	AAC	ATG	ACA	GAG	GAC	CAT	GTG	433
Pro	Met	Gln	Asn	Ala	Thr	Lys	Tyr	Gly	Asn	Met	Thr	Glu	Asp	His	Val	
			130					135					140			
ATG	CAC	CTG	CTC	CAG	AAT	GCT	GAC	CCC	CTG	AAG	GTG	TAC	CCG	CCA	CTG	481
Met	His	Leu	Leu	Gln	Asn	Ala	Asp	Pro	Leu	Lys	Val	Tyr	Pro	Pro	Leu	
		145					150					155				
AAG	GGG	AGC	TTC	CCG	GAG	AAC	CTG	AGA	CAC	CTT	AAG	AAC	ACC	ATG	GAG	529
Lys	Gly	Ser	Phe	Pro	Glu	Asn	Leu	Arg	His	Leu	Lys	Asn	Thr	Met	Glu	
	160					165					170					
ACC	ATA	GAC	TGG	AAG	GTC	TTT	GAG	AGC	TGG	ATG	CAC	CAT	TGG	CTC	CTG	577
Thr	Ile	Asp	Trp	Lys	Val	Phe	Glu	Ser	Trp	Met	His	His	Trp	Leu	Leu	
175					180					185					190	

FIGURE 12

TTT GAA ATG AGC AGG CAC TCC TTG GAG CAA AAG CCC ACT GAC GCT CCA	625
Phe Glu Met Ser Arg His Ser Leu Glu Gln Lys Pro Thr Asp Ala Pro	
195 200 205	
CCG AAA GAG TCA CTG GAA CTG GAG GAC CCG TCT TCT GGG CTG GGT GTG	673
Pro Lys Glu Ser Leu Glu Leu Glu Asp Pro Ser Ser Gly Leu Gly Val	
210 215 220	
ACC AAG CAG GAT CTG GGC CCA GTC CCC ATG TGAGAGCAGC AGAGGCGGTC	723
Thr Lys Gln Asp Leu Gly Pro Val Pro Met	
225 230	

FIGURE 12 Continued

CCGCCTCGGC	ATG GCG CCC CGC AGC GCC CGG CGA CCC CTG CTG CTG CTA	229
	Met Ala Pro Arg Ser Ala Arg Arg Pro Leu Leu Leu Leu	
	1 5 10	
CTG CCT GTT GCT GCT GCT CGG CCT CAT GCA TTG TCG TCA GCA GCC ATG	277	
Leu Pro Val Ala Ala Ala Arg Pro His Ala Leu Ser Ser Ala Ala Met		
15 20 25		
TTT ATG GTG AAA AAT GGC AAC GGG ACC GCG TGC ATA ATG GCC AAC TTC	325	
Phe Met Val Lys Asn Gly Asn Gly Thr Ala Cys Ile Met Ala Asn Phe		
30 35 40 45		
TCT GCT GCC TTC TCA GTG AAC TAC GAC ACC AAG AGT GGC CCC AAG AAC	373	
Ser Ala Ala Phe Ser Val Asn Tyr Asp Thr Lys Ser Gly Pro Lys Asn		
50 55 60		
ATG ACC TTT GAC CTG CCA TCA GAT GCC ACA GTG GTG CTC AAC CGC AGC	421	
Met Thr Phe Asp Leu Pro Ser Asp Ala Thr Val Val Leu Asn Arg Ser		
65 70 75		
TCC TGT GGA AAA GAG AAC ACT TCT GAC CCC AGT CTC GTG ATT GCT TTT	469	
Ser Cys Gly Lys Glu Asn Thr Ser Asp Pro Ser Leu Val Ile Ala Phe		
80 85 90		
GGA AGA GGA CAT ACA CTC ACT CTC AAT TTC ACG AGA AAT GCA ACA CGT	517	
Gly Arg Gly His Thr Leu Thr Leu Asn Phe Thr Arg Asn Ala Thr Arg		
95 100 105		
TAC AGC GTT CAG CTC ATG AGT TTT GTT TAT AAC TTG TCA GAC ACA CAC	565	
Tyr Ser Val Gln Leu Met Ser Phe Val Tyr Asn Leu Ser Asp Thr His		
110 115 120 125		
CTT TTC CCC AAT GCG AGC TCC AAA GAA ATC AAG ACT GTG GAA TCT ATA	613	
Leu Phe Pro Asn Ala Ser Ser Lys Glu Ile Lys Thr Val Glu Ser Ile		
130 135 140		
ACT GAC ATC AGG GCA GAT ATA GAT AAA AAA TAC AGA TGT GTT AGT GGC	661	
Thr Asp Ile Arg Ala Asp Ile Asp Lys Lys Tyr Arg Cys Val Ser Gly		
145 150 155		
ACC CAG GTC CAC ATG AAC AAC GTG ACC GTA ACG CTC CAT GAT GCC ACC	709	
Thr Gln Val His Met Asn Asn Val Thr Val Thr Leu His Asp Ala Thr		
160 165 170		
ATC CAG GCG TAC CTT TCC AAC AGC AGC TTC AGC AGG GGA GAG ACA CGC	757	
Ile Gln Ala Tyr Leu Ser Asn Ser Ser Phe Ser Arg Gly Glu Thr Arg		
175 180 185		

FIGURE 13

TGT GAA CAA GAC AGG CCT TCC CCA ACC ACA GCG CCC CCT GCG CCA CCC Cys Glu Gln Asp Arg Pro Ser Pro Thr Thr Ala Pro Pro Ala Pro Pro 190 195 200 205	805
AGC CCC TCG CCC TCA CCC GTG CCC AAG AGC CCC TCT GTG GAC AAG TAC Ser Pro Ser Pro Ser Pro Val Pro Lys Ser Pro Ser Val Asp Lys Tyr 210 215 220	853
AAC GTG AGC GGC ACC AAC GGG ACC TGC CTG CTG GCC AGC ATG GGG CTG Asn Val Ser Gly Thr Asn Gly Thr Cys Leu Leu Ala Ser Met Gly Leu 225 230 235	901
CAG CTG AAC CTC ACC TAT GAG AGG AAG GAC AAC ACG ACG GTG ACA AGG Gln Leu Asn Leu Thr Tyr Glu Arg Lys Asp Asn Thr Thr Val Thr Arg 240 245 250	949
CTT CTC AAC ATC AAC CCC AAC AAG ACC TCG GCC AGC GGG AGC TGC GGC Leu Leu Asn Ile Asn Pro Asn Lys Thr Ser Ala Ser Gly Ser Cys Gly 255 260 265	997
GCC CAC CTG GTG ACT CTG GAG CTG CAC AGC GAG GGC ACC ACC GTC CTG Ala His Leu Val Thr Leu Glu Leu His Ser Glu Gly Thr Thr Val Leu 270 275 280 285	1045
CTC TTC CAG TTC GGG ATG AAT GCA AGT TCT AGC CGG TTT TTC CTA CAA Leu Phe Gln Phe Gly Met Asn Ala Ser Ser Ser Arg Phe Phe Leu Gln 290 295 300	1093
GGA ATC CAG TTG AAT ACA ATT CTT CCT GAC GCC AGA GAC CCT GCC TTT Gly Ile Gln Leu Asn Thr Ile Leu Pro Asp Ala Arg Asp Pro Ala Phe 305 310 315	1141
AAA GCT GCC AAC GGC TCC CTG CGA GCG CTG CAG GCC ACA GTC GGC AAT Lys Ala Ala Asn Gly Ser Leu Arg Ala Leu Gln Ala Thr Val Gly Asn 320 325 330	1189
TCC TAC AAG TGC AAC GCG GAG GAG CAC GTC CGT GTC ACG AAG GCG TTT Ser Tyr Lys Cys Asn Ala Glu Glu His Val Arg Val Thr Lys Ala Phe 335 340 345	1237
TCA GTC AAT ATA TTC AAA GTG TGG GTC CAG GCT TTC AAG GTG GAA GGT Ser Val Asn Ile Phe Lys Val Trp Val Gln Ala Phe Lys Val Glu Gly 350 355 360 365	1285
GGC CAG TTT GGC TCT GTG GAG GAG TGT CTG CTG GAC GAG AAC AGC ACG Gly Gln Phe Gly Ser Val Glu Glu Cys Leu Leu Asp Glu Asn Ser Thr 370 375 380	1333

FIGURE 13. CONTINUED

CTG ATC CCC ATC GCT GTG GGT GGT GCC CTG GCG GGG CTG GTC CTC ATC	1381
Leu Ile Pro Ile Ala Val Gly Gly Ala Leu Ala Gly Leu Val Leu Ile	
385 390 395	
GTC CTC ATC GCC TAC CTC GTC GGC AGG AAG AGG AGT CAC GCA GGC TAC	1429
Val Leu Ile Ala Tyr Leu Val Gly Arg Lys Arg Ser His Ala Gly Tyr	
400 405 410	
CAG ACT ATC TAGCCTGGTG CACGCAGGCA CAGCAGCTGC AGGGGCCTCT	1478
Gln Thr Ile	
415	

FIGURE 13 CONTINUED

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
ATGATCACATTTCCTGCCGCTGCTGCTGGGGCTCAGCCTGGGCTGCACAGGAGCAGGTGGCTTCGTGGCCC
TACTAGTGTAAGGACGGCGACGACGACCCCGAGTCGGACCCGACGTGTCCTCGTCCACCGAAGCACCGGG
M I T F L P L L G L S L G C T G A G G F V A>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
ATGTGGAAAGCACCTGTCTGTTGGATGATGCTGGGACTCCAAAGGATTTACATACTGCATCTCCTTCAA
TACACCTTTTCGTGGACAGACAACCTACTACGACCCTGAGGTTTCCTAAAGTGTATGACGTAGAGGAAGTT
E V E S T C L L D D A G T P K D F T Y C I S F N>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
CAAGGATCTGCTGACCTGCTGGGATCCAGAGGAGAATAAGATGGCCCCCTTGCGAATTTGGGGTGCTGAAT
GTTCCCTAGACGACTGGACGACCCTAGGTCTCCTCTTATTCTACCGGGGAACGCTTAAACCCACGACTTA
K D L L T C W D P E E N K M A P C E F G V L N>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
AGCTTGGCGAATGTCTCTCACAGCACCTCAACCAAAAAGACACCCTGATGCAGCGCTTGCGCAATGGGC
TCGAACCGCTTACAGGAGASTGTCTGGAGTTGGTTTTTCTGTGGGACTACGTGCGGAACCGGTTACCCG
S L A N V L S Q H L N Q K D T L M Q R L R N G>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
TTCAGAATTGTGCCACACACACCCAGCCCTTCTGGGGATCACTGACCAACAGGACACGGCCACCATCTGT
AAGTCTTAACACGGTGTGTGTGGGTGCGGAAGACCCCTAGTGACTGGTTGTCTGTGCCGGTGGTAGACA
L Q N C A T E T Q P F W G S L T N R T R P P S V>

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
GCAAGTAGCCAAAACCACTCCTTTTAACACGAGGGAGCCTGTGATGCTGGCCTGCTATGTGTGGGGCTTC
CGTTCATCGGTTTTTGGTGAGGAAAATTGTGCTCCCTCGGACACTACGACCGGACGATACACACCCCGAAG
Q V A K T T P F N T R E P V M L A C Y V W G F>

      430      440      450      460      470      480      490
      *      *      *      *      *      *      *
TATCCAGCAGAAGTGACTATCACTGGAGGAAGAACGGGAAGCTTGTGATGCCTCACAGCAGTGGCGACA
ATAGGTGCTCTTCACTGATAGTGACCTCCTTCTTGGCCTTGAACAGTACGGAGTGTGCTCACGCGTGT
Y P A E V T E T W R K N G K L V M P H S S A H>

      500      510      520      530      540      550      560
      *      *      *      *      *      *      *
AGACTGCCCGAGCCCAATGGAGACTGGACATACCAGACCCTCTCCCATTTAGCCTTAACCCCTCTTACGG
TCTGACGGGTGCGGTTACCTCTGACCTGTATGGTCTGGGAGAGGGTAAATCGGAATTGGGGGAGAATGCC
K T A Q P N G D W T Y Q T L S H L A L T P S Y G>

```

FIGURE 14

```

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *
GGACACTTACACCTGTGTGGTAGAGCACATTGGGSGTCCTGAGCCCATCCTTCGGGACTGGACACCTGGG
CCTGTGAATGTGGACACACCATCTCGTGTAAACCCGAGGACTCGGGTAGGAAGCCCTGACCTGTGGACCC
  D T Y T C V V E H I G A P E P I L R D W T P G>

      640      650      660      670      680      690      700
      *      *      *      *      *      *      *
CTGTCCCCCATGCAGACCCCTGAAGGTTTCTGTGTCTGCAGTGACTCTGGGCCTGGGCCTCATCATCTTCT
GACAGGGGGTACGTCTGGGACTTCCAAAGACACAGACGTCAGTGAGACCCGGACCCGGAGTAGTAGAAGA
  L S P M Q T L K V S V S A V T L G L G L I I F>

      710      720      730      740      750      760      770
      *      *      *      *      *      *      *
CTCTTGGTGTGATCAGCTGGCGGAGAGCTGGCCACTCTAGTTACACTCCTCTTCCTGGGTCCAATTATTC
GAGAACCACTAGTCGACCGCCTCTCGACCGGTGAGATCAATGTGAGGAGAAGGACCCAGGTTAATAAG
  S L G V I S W R R A G H S S Y T P L P G S N Y S>

      780      790
      *      *
AGAAGGATGGCACATTTCTAG
TCTTCCTACCGTGTAAGGATC
  E G W H I S *>

```

FIGURE 14 Continued

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
ATGGGTTCTGGGTGGGTCCCCTGGGTGGGTCTGCTAGTGAATCTGACCCAACTGGATTCTCCATGA
TACCCAAGACCCACCCAGGGGACCCACCACCGAGACGATCACTTAGACTGGGTTGACCTAAGGAGGTACT
M G S G W V P W V V A L L V N L T Q L D S S M>

      80      90     100     110     120     130     140
      *      *      *      *      *      *      *
CTCAAGGCACAGACTCTCCAGAAGATTTTGTGATTCAGGCAAAGGCTGACTGTTACTTCACCAACGGGAC
GAGTTCCGTGTCTGAGAGGTCTTCTAAACACTAAGTCCGTTTCCGACTGACAATGAAGTGGTTGCCCTG
T Q G T D S P E D F V I Q A K A D C Y F T N G T>

     150     160     170     180     190     200     210
      *      *      *      *      *      *      *
AGAAAAGGTGCAGTTTGTGGTCAGATTCATCTTTAACTGGAGGAGTATGTACGTTTCGACAGTGATGTG
TCTTTTCCACGTCAAACACCAAGTCTAAGTAGAAATTGAACCTCCTCATACATGCAAAGCTGTCACTACAC
E K V Q F V V R F I F N L E E Y V R F D S D V>

     220     230     240     250     260     270     280
      *      *      *      *      *      *      *
GGGATGTTTGTGGCATTGACCAAGCTGGGGCAGCCAGATGCTGAGCAGTGGAACAGCCGGCTGGATCTCT
CCCTACAAACACCGTAAGTGGTTGACCCCGTCGGTCTACGACTCGTCACTTGTGCGGCCGACCTAGAGA
G M F V A L T K L G Q P D A E Q W N S R L D L>

     290     300     310     320     330     340     350
      *      *      *      *      *      *      *
TGGAGAGGAGCAGACAGGCCGTGGATGGGGTCTGTAGACACAACACTACAGGCTGGGCGCACCCCTTCACTGT
ACCTCTCCTCGTCTGTCCGGCACCTACCCAGACATCTGTGTTGATGTCCGACCCGCGTGGGAAGTGACA
L E R S R Q A V D G V C R H N Y R L G A P F T V>

     360     370     380     390     400     410     420
      *      *      *      *      *      *      *
GGGGAGAAAAGTGCAACCAGAGGTGACAGTGTACCCAGAGAGGACCCCACTCCTGCACCAGCATAATCTG
CCCCCTCTTTTCACGTTGGTCTCCACTGTCACATGGGTCTCTCCTGGGGTGAGGACGTGGTCTATTAGAC
G R X V Q P E V T V Y P E R T P L L H Q H N L>

     430     440     450     460     470     480     490
      *      *      *      *      *      *      *
CTGCACTGCTCTGTGACAGGCTTCTATCCAGGGGATATCAAGATCAAGTGGTTCCTGAATGGGCAGGAGG
GACGTGACGAGACACTGTCCGAAGATAGGTCCCCTATAGTTCTAGTTCACCAAGGACTTACCCGTCCTCC
L H C S V T G F Y P G D I K I K W F L N G Q E>

     500     510     520     530     540     550     560
      *      *      *      *      *      *      *

```

FIGURE 15

AGAGAGCTGGGGTCATGTCCACTGGCCCTATCAGGAATGGAGACTGGACCTTTCAGACTGTGGTGATGCT
TCTCTCGACCCCACTACAGGTGACCGGGATAGTCCTTACCTCTGACCTGGAAAGTCTGACACCACTACGA
E R A G V M S T G P I R N G D W T F Q T V V M L>

570 580 590 600 610 620 630
* * * * *
AGAAATGACTCCTGAACTTGGACATGTCTACACCTGCCTTGTGATCACTCCAGCCTGCTGAGCCCTGTT
TCTTTACTGAGGACTTGAACCTGTACAGATGTGGACGGAACAGCTAGTGAGGTCGGACGACTCGGGACAA
E M T P E L G H V Y T C L V D H S S L L S P V>

640 650 660 670 680 690 700
* * * * *
TCTGTGGAGTGGAGAGCTCAGTCTGAATATTCTTGGAGAAAGATGCTGAGTGGCATTGCAGCCTTCCTAC
AGACACCTCACCTCTCGAGTCAGACTTATAAGAACCTCTTTCTACGACTCACCGTAACGTCGGAAGGATG
S V E W R A Q S E Y S W R K M L S G I A A F L>

710 720 730 740 750 760 770
* * * * *
TTGGGCTAATCTTCCTTCTGGTGCGAATCGTCATCCAGCTAAGGGCTCAGAAAGGATATGTGAGGACGCA
AAGCCGATTAGAAGGAAGACCAACCTTAGCAGTAGGTGCGATTCCCGAGTCTTTCCTATACACTCCTGCGT
L G L I F L L V G I V I Q L R A Q K G Y V R T Q>

780 790 800 810 820
* * * * *
GATGTCTGGTAATGAGGTCTCAAGAGCTGTTCTGCTCCCTCAGTCATGCTAA
CTACAGACCATTACTCCAGAGTTCTCGACAAGACGAGGGAGTCAGTACGATT
M S G N E V S R A V L L P Q S C *>

FIGURE 15 CONTINUED

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
ATGCCTGGGGGTCCAGGAGTCCCTCCAAGCTCTGCCTGCCACCATCTTCCTCCTCTTCCTGCTGTCTGCTG
TACGGACCCCCAGGTCCCTCAGGAGGTTCCGAGACGGACGGTGGTAGAAGGAGGAGAAGGACGACAGACGAC
M P G G P G V L Q A L P A T I F L L F L L S A>

      80      90      100     110     120     130     140
      *      *      *      *      *      *      *
TCTACCTGGGCCCTGGGTGCCAGGCCCTGTGGATGCACAAGGTCCCAGCATCATTGATGGTGAGCCTGGG
AGATGGACCCCGGGACCCACGGTCCGGGACACCTACGTGTTCCAGGGTCTGTAGTAACCTACCACTCGGACCC
V Y L G P G C Q A L W M H K V P A S L M V S L G>

      150     160     170     180     190     200     210
      *      *      *      *      *      *      *
GGAAGACGCCCCACTTCCAATGCCCGCACAAATAGCAGCAACAACGCCAACGTACCTGGTGGCGCGTCCCTC
CCTTCTGCGGGTGAAGGTTACGGGCGTGTATCGTCGTTGTTGCGGTTGCAGTGGACCACCGCGCAGGAG
E D A H F C C P H N S S N N A N V T W W R V L>

      220     230     240     250     260     270     280
      *      *      *      *      *      *      *
CATGGCAACTACACGTGGCCCCCTGAGTTCTTGGGCCCCGGGCGAGGACCCCAATGGTACGCTGATCATCC
GTACCGTTGATGTGCACCCGGGGACTCAAGAACCCGGGCCCCGCTCCTGGGGTTACCATGCGACTAGTAGG
H G N Y T W P P E F L G P G E D P N G T L I I>

      290     300     310     320     330     340     350
      *      *      *      *      *      *      *
AGAATGTGAACAAGAGCCATGGGGGCATATACGTGTGCCGGGTCCAGGAGGGCAACGAGTCATACCAGCA
TCTTACACTTGTTCTCGGTACCCCCGTATATGCACACGGCCCCAGGTCCCTCCCGTTGCTCAGTATGGTCGT
Q N V N K S E G G I Y V C R V Q E G N E S Y Q Q>

      360     370     380     390     400     410     420
      *      *      *      *      *      *      *
GTCCTGCGGCACCTACCTCCGCGTGCGGCCAGCCGCCCCCAGGCCCTTCCTGGACATGGGGGAGGGCACC
CAGGACGCCCGTGGATGGAGGCGCACGCGGTCCGGCGGGGGTCCGGGAAGGACCTGTACCCCCCTCCCGTGG
S C G T Y L R V R Q P P P R P F L D M G E G T>

      430     440     450     460     470     480     490
      *      *      *      *      *      *      *
AAGAACCGAATCATCACACCCGAGGGGATCATCCTCCTGTTCTGCGCGGTGGTGCCTGGGACGCTGCTGC
TTCTTGGCTTAGTAGTGTGGCTCCCTAGTAGGAGGACAAGACGCGCCACCACGGACCCTGCGACGACG
K N R I I T A E G I I L L F C A V V P G T L L>

```

FIGURE 16

```
      500      510      520      530      540      550      560
      *      *      *      *      *      *      *      *
TGTTTCAGGAAACGATGGCAGAACGAGAAGCTCGGGTTGGATGCCGGGGATGAATATGAAGATGAAAACCT
ACAAGTCCTTTGCTACCGTCTTGCTCTTCGAGCCCAACCTACGGCCCCCTACTTATACTTCTACTTTTGGA
L F R K R W Q N E K L G L D A G D E Y E D E N L>

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *      *
TTATGAAGGCCTGAACCTGGACGACTGCTCCATGTATGAGGACATCTCCCGGGGCCTCCAGGGCACCTAC
AATACTTCCGGACTTGGACCTGCTGACGAGGTACATACTCCTGTAGAGGGCCCCGGAGGTCCCGTGGATG
Y E G L N L D D C S M Y E D I S R G L Q G T Y>

      640      650      660      670      680      690      700
      *      *      *      *      *      *      *      *
CAGGATGTGGGCAGCCTCAACATAGGAGATGTCCAGCTGGAGAAGCCGTGACACCCCTACTCCTGCCAGG
GTCCTACACCCGTCCGAGTTGTATCCTCTACAGGTCGACCTCTTCGGCACTGTGGGGATGAGGACGGTCC
Q D V G S L N I G D V Q L E K P *>
```

FIGURE 16 CONTINUED

GAATTCGCG GTGACC ATG GCC AGG CTG GCG TTG TCT CCT GTG CCC AGC	49
Met Ala Arg Leu Ala Leu Ser Pro Val Pro Ser	
1 5 10	
CAC TGG ATG GTG GCG TTG CTG CTG CTG CTC TCA GCT GAG CCA GTA CCA	97
His Trp Met Val Ala Leu Leu Leu Leu Leu Ser Ala Glu Pro Val Pro	
15 20 25	
GCA GCC AGA TCG GAG GAC CGG TAC CGG AAT CCC AAA GGT AGT GCT TGT	145
Ala Ala Arg Ser Glu Asp Arg Tyr Arg Asn Pro Lys Gly Ser Ala Cys	
30 35 40	
TCG CGG ATC TGG CAG AGC CCA CGT TTC ATA GCC AGG AAA CGG CGC TTC	193
Ser Arg Ile Trp Gln Ser Pro Arg Phe Ile Ala Arg Lys Arg Arg Phe	
45 50 55	
ACG GTG AAA ATG CAC TGC TAC ATG AAC AGC GCC TCC GGC AAT GTG AGC	241
Thr Val Lys Met His Cys Tyr Met Asn Ser Ala Ser Gly Asn Val Ser	
60 65 70 75	
TGG CTC TGG AAG CAG GAG ATG GAC GAG AAT CCC CAG CAG CTG AAG CTG	289
Trp Leu Trp Lys Gln Glu Met Asp Glu Asn Pro Gln Gln Leu Lys Leu	
80 85 90	
GAA AAG GGC CGC ATG GAA GAG TCC CAG AAC GAA TCT CTC GCC ACC CTC	337
Glu Lys Gly Arg Met Glu Glu Ser Gln Asn Glu Ser Leu Ala Thr Leu	
95 100 105	
ACC ATC CAA GGC ATC CGG TTT GAG GAC AAT GGC ATC TAC TTC TGC CAG	385
Thr Ile Gln Gly Ile Arg Phe Glu Asp Asn Gly Ile Tyr Phe Cys Gln	
110 115 120	
CAG AAG TGC AAC AAC ACC TCG GAG GTC TAC CAG GGC TGC GGC ACA GAG	433
Gln Lys Cys Asn Asn Thr Ser Glu Val Tyr Gln Gly Cys Gly Thr Glu	
125 130 135	
CTG CGA GTC ATG GGA TTC AGC ACC TTG GCA CAG CTG AAG CAG AGG AAC	481
Leu Arg Val Met Gly Phe Ser Thr Leu Ala Gln Leu Lys Gln Arg Asn	
140 145 150 155	
ACG CTG AAG GAT GGT ATC ATC ATG ATC CAG ACG CTG CTG ATC ATC CTC	529
Thr Leu Lys Asp Gly Ile Ile Met Ile Gln Thr Leu Leu Ile Ile Leu	
160 165 170	

FIGURE 17

TTC ATC ATC GTG CCT ATC TTC CTG CTG CTG GAC AAG GAT GAC AGC AAG	577
Phe Ile Ile Val Pro Ile Phe Leu Leu Leu Asp Lys Asp Asp Ser Lys	
175 180 185	
GCT GGC ATG GAG GAA GAT CAC ACC TAC GAG GGC CTG GAC ATT GAC CAG	625
Ala Gly Met Glu Glu Asp His Thr Tyr Glu Gly Leu Asp Ile Asp Gln	
190 195 200	
ACA GCC ACC TAT GAG GAC ATA GTG ACG CTG CGG ACA GGG GAA GTG AAG	673
Thr Ala Thr Tyr Glu Asp Ile Val Thr Leu Arg Thr Gly Glu Val Lys	
205 210 215	
TGG TCT GTA GGT GAG CAC CCA GGC CAG GAG TGACAGCCAG GTCGCCCCAT	723
Trp Ser Val Gly Glu His Pro Gly Gln Glu	
220 225 230	

FIGURE 17 CONTINUED

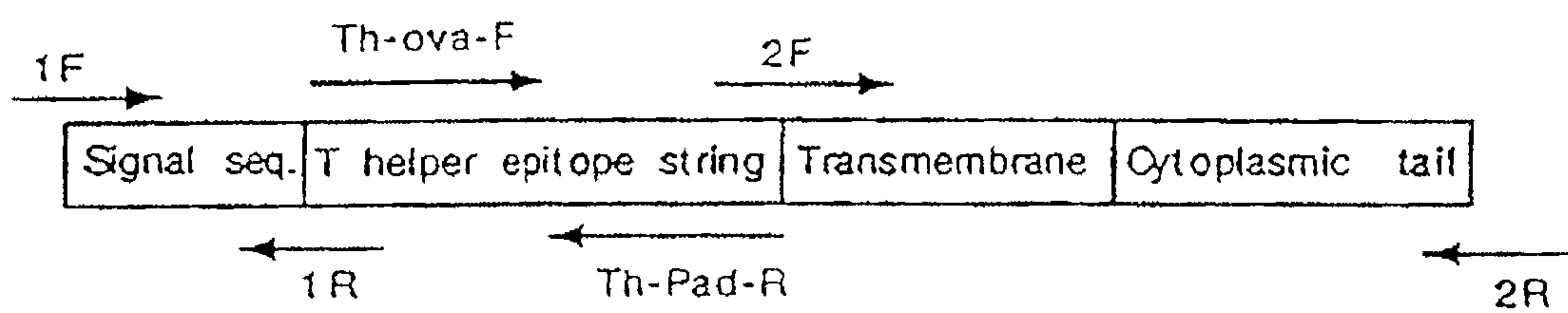


FIGURE 18

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      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GACCGATCGGGAGATCTCCCGATCCCCTATGGTCCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTT
CTGCCTAGCCCTCTAGAGGGCTAGGGGATAACCAGCTGAGAGTCATGTTAGACGAGACTACGGCGTATCAA

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
AAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCCGTGAGTAGTCCGCGAGCAAAATTTAAGCTACA
TTCGGTCATAGACGAGGGACGAACACACAACCTCCAGCGACTCATCACGCGCTCGTTTTAAATTCGATGT

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
ACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCG
TGTTCGGTTCGGAAGTGGCTGTTAACGTAATTCTTAGACGAATCCCAATCCGCAAAACGCGACGAAGCGC

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
ATGTACGGGCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTTC
TACATGCCCCGGTCTATATGCGCAACTGTAATAATAACTGATCAATAATTATCATTAGTTAATGCCCCAG

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
ATTAGTTCATAGCCCATATATGGAGTTCGCGGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCG
TAATCAAGTATCGGGTATATACCTCAAGGCGCAATGTATTGAATGCCATTTACCGGGCGGACCGACTGGC

      360      370      380      390      400      410      420
      *      *      *      *      *      *      *
CCCAACGACCCCCGCCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
GGGTTGCTGGGGGCGGTAAGTGCAGTTATTACTGCATACAAGGCTATCATTGCGGTTATCCCTGAAAGG

      430      440      450      460      470      480      490
      *      *      *      *      *      *      *
ATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCC
TAACTGCAGTTACCCACCTGATAAATGCCATTTGACGGGTGAACCGTCATGTAGTTACATAGTATACGG

      500      510      520      530      540      550      560
      *      *      *      *      *      *      *
AAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTA
TTCATGCGGGGGATAACTGCAGTTACTGCCATTTACCGGGCGGACCGTAATACGGGTCATGTACTGGAAT

      570      580      590      600      610      620      630
      *      *      *      *      *      *      *
TGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGC
ACCCTGAAAGGATGAACCGTCATGTAGATGCATAATCAGTAGCGATAATGGTACCCTACGCCAAAACCG

      640      650      660      670      680      690      700
      *      *      *      *      *      *      *
AGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAA
TCATGTAGTTACCCGCACCTATCGCCAACTGAGTGCCCCTAAAGGTTTCAGAGGTGGGGTAACTGCAGTT

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FIGURE 19

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      710      720      730      740      750      760      770
      *      *      *      *      *      *      *
TGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACTCCGCCCCATTGACG
ACCCCTCAAACAAAACCGTGGTTTTAGTTGCCCTGAAAGGTTTACAGCATTGTTGAGGCGGGGTAAGTGC

      780      790      800      810      820      830      840
      *      *      *      *      *      *      *
CAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCA
GTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGAGAGACCGATTGATCTCTTGGGT

      850      860      870      880      890      900      910
      *      *      *      *      *      *      *
CTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGAGTAAGTACCG
GACGAATGACCGAATAGCTTTAATTATGCTGAGTGATATCCCTCTGGCTTCGACCGATCTCATTGATGGC

      920      930      940      950      960      970      980
      *      *      *      *      *      *      *
CCTATAGAGTCTATAGGCCCAACCCCTTGGCTTCTTATGCATGCTATACTGTTTTTGGCTTGGGGTCTAT
GGATATCTCAGATATCCGGGTGGGGGAACCGAAGATACGTACGATATGACAAAACCGAACCCAGATA

      990      1000      1010      1020      1030      1040      1050
      *      *      *      *      *      *      *
ACACCCCGCTTCTCATGTTATAGGTGATGGTATAGCTTAGCCTATAGGTGTGGGTTATTGACCATTAT
TGTGGGGGCGAAGGAGTACAATATCCACTACCATATCGAATCGGATATCCACACCCAATAACTGGTAATA

      1060      1070      1080      1090      1100      1110      1120
      *      *      *      *      *      *      *
TGACCACTCCCTATTGGTGACGATACTTTCCATTACTAATCCATAACATGGCTCTTTGCCACAACCTCTC
ACTGGTGAGGGGATAACCACTGCTATGAAAGGTAATGATTAGGTATTGTACCGAGAAACGGTGTGAGAG

      1130      1140      1150      1160      1170      1180      1190
      *      *      *      *      *      *      *
TTTATTGGCTATATGCCAATACACTGTCTTCAGAGACTGACACGGACTCTGTATTTTACAGGATGGGG
AAATAACCGATATACGGTTATGTGACAGGAAGTCTCTGACTGTGCCTGAGACATAAAAATGTCTTACCCC

      1200      1210      1220      1230      1240      1250      1260
      *      *      *      *      *      *      *
TCTCATTTATTATTTACAAATTCACATATACAACACCACCGTCCCCAGTGCCCGCAGTTTTTTATTAAACA
AGAGTAAATAATAAATGTTTAASTGTATATGTTGTGGTGGCAGGGGTCACGGGCGTCAAAAATAATTTGT

      1270      1280      1290      1300      1310      1320      1330
      *      *      *      *      *      *      *
TAACGTGGGATCTCCACGCGAATCTCGGGTACGTGTTCCGGACATGGGCTCTTCTCCGGTAGCGGCGGAG
ATTGCACCCTAGAGGTGGGCTTAGAGCCCATGCACAAGGCCTGTACCCGAGAAGAGGCCATCGCCGCCTC

      1340      1350      1360      1370      1380      1390      1400
      *      *      *      *      *      *      *
CTTCTACATCCGAGCCCTGCTCCCATGCCTCCAGCGACTCATGGTCGCTCGGCAGCTCCTTGCTCCTAAC
GAAGATGTAGGCTCGGGACGAGGGTACGGAGGTGCTGAGTACCAGCGAGCCGTCGAGGAACGAGGATTG

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FIGURE 19 CONTINUED

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      1410      1420      1430      1440      1450      1460      1470
      *      *      *      *      *      *      *
AGTGGAGGCCAGACTTAGGCACAGCAGATGCCCACCACCACCAGTGTGCCGCACAAGGCCGTGGCGGTA
TCACCTCCGGTCTGAATCCGTGTCGTGCTACGGGTGGTGGTGGTGCACACGGCGTGTTCGGGCACCGCCAT

      1480      1490      1500      1510      1520      1530      1540
      *      *      *      *      *      *      *
GGGTATGTGTCTGAAAATGAGCTCGGGGAGCGGGCTTGACCGCTGACGCATTTGGAAGACTTAAGGCAG
CCCATACACAGACTTTTACTCGAGCCCTCGCCCGAACGTGGCGACTGCGTAAACCTTCTGAATTCGGTC

      1550      1560      1570      1580      1590      1600      1610
      *      *      *      *      *      *      *
CGGCAGAAGAAGATGCAGGCAGCTGAGTTGTTGTGTTCTGATAAGAGTCAGAGGTAACCTCCCGTTGCGGT
GCCGTCTTCTTCTACGTCCGTGACTCAACAACACAGACTATTCTCAGTCTCCATTGAGGGCAACGCCA

      1620      1630      1640      1650      1660      1670      1680
      *      *      *      *      *      *      *
GCTGTTAACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGCCGCGCGCGCCACCAGACATAAT
CGACAATTGCCACCTCCCGTCACATCAGACTCGTCATGAGCAACGACGGCGCGCGCGGTGGTCTGTATTA

      1690      1700      1710      1720      1730      1740      1750
      *      *      *      *      *      *      *
AGCTGACAGACTAACAGACTGTTCCCTTCCATGGGTCTTTTCTGCAGGCTAGCCGGCCTGAATTCGGATA
TCGACTGTCTGATGTCTGACAAGGAAAGGTACCCAGAAAAGACGTCCGATCGGCCGGACTTAAGCCTAT

      1760      1770      1780      1790      1800      1810      1820
      *      *      *      *      *      *      *
TCCAAGCTTGATGAATAAAAGATCAGAGCTCTAGTGATCTGTGTGTTGGTTTTTTGTGTGCTCGAGCCCC
AGGTTGGAAGTACTTATTTCTAGTCTCGAGATCACTAGACACACAAACAAAAACACACGAGCTCGGGG

      1830      1840      1850      1860      1870      1880      1890
      *      *      *      *      *      *      *
AGCTGGTTCTTTCCGCCCTCAGAAGCCATAGAGCCACCGCATCCCAGCATGCCGTGCTATTGTCTTCCCA
TCGACCAAGAAAGCGGAGTCTTCGGTATCTCGGGTGGCGTAGGGGTGCTACGGACGATAACAGAAGGGT

      1900      1910      1920      1930      1940      1950      1960
      *      *      *      *      *      *      *
ATCCTCCCCCTTGCTGTCTGCCCCACCCACCCCCAGAAATAGAATGACACCTACTCAGACAATGCGAT
TAGGAGGGGGAACGACAGGACGGGGTGGGGTGGGGGTCTTATCTTACTGTGGATGAGTCTGTTACGCTA

      1970      1980      1990      2000      2010      2020      2030
      *      *      *      *      *      *      *
GCAATTTCTCTATTTTATTAGGAAAGGACAGTGGGAGTGGCACCTTCCAGGGTCAAGGAAGGCACGGGGG
CGTTAAAGGAGTAAAATAATCCTTTCCTGTACCCCTCACCGTGAAGGTCCCAGTTCCTTCCGTGCCCCC

      2040      2050      2060      2070      2080      2090      2100
      *      *      *      *      *      *      *
AGGGGCAACAACAGATGGCTGGCAACTAGAAGGCACAGTGGAGGCTGATCAGCGAGCTCTAGCGGTACC
TCCCCGTTTGTGTCTACCGACCGTTGATCTTCGGTGTGAGTCCGACTAGTCCGCTCGAGATCGCCATGG

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FIGURE 19 CONTINUED

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      2110      2120      2130      2140      2150      2160      2170
      *      *      *      *      *      *      *
GGCATTAGTCTATGGCCGACTCTAGATTTTCTCCTTGCGGCCGCCCTAGATGCATGCTCGATCGACCTGC
CCGTAATCAGATAACCGGCTGAGATCTAAAAGAGGAACGCCGCGGGATCTACGTACGAGCTAGCTGGACG

      2180      2190      2200      2210      2220      2230      2240
      *      *      *      *      *      *      *
AGTTGGACCTGGGAGTGGACACCTGTGGAGAGAAAGGCAAAGTGGATGTCATTGTCACTCAAGTGTATGG
TCAACCTGGACCCCTCACCTGTGGACACCTCTCTTTCCGTTTCACCTACAGTAACAGTGAGTTCACATACC

      2250      2260      2270      2280      2290      2300      2310
      *      *      *      *      *      *      *
CCAGATCTCAAGCCTGCCACACCTCAAGCTAGCTTGACAACAAAAAGATTGTCTTTTCTGACCAGATGGA
GGTCTAGAGTTTCGGACGGTGTGGAGTTCGATCGAACTGTTGTTTTCTAACAGAAAAGACTGGTCTACCT

      2320      2330      2340      2350      2360      2370      2380
      *      *      *      *      *      *      *
CGCGGCCACCCCTCAAAGGCATCACCGCGGCCAGGTGAATATCAAATCCTCCTCGTTTTTGGAAACTGAC
GCGCCGGTGGGAGTTTCCGTAGTGGCGCCCGGTCCACTTATAGTTTAGGAGGAGCAAAAACCTTTGACTG

      2390      2400      2410      2420      2430      2440      2450
      *      *      *      *      *      *      *
AATCTTAGCGCAGAAGTCATGCCCGCTTTTGAGAGGGAGTACTCACCCCAACAGCTGGCCCTCGCAGACA
TTAGAATCGCGTCTTCAGTACGGGCGAAACTCTCCTCATGAGTGGGGTTGTGACCGGGAGCGTCTGT

      2460      2470      2480      2490      2500      2510      2520
      *      *      *      *      *      *      *
GCGAATTAATTCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGCAAGCTAGCTTGGGTCTCCC
CGCTTAATTAAGGTGCTGTGACCGCCGGCAATGATCACCTAGGCTCGAGCGTTCGATCGAACCCAGAGGG

      2530      2540      2550      2560      2570      2580      2590
      *      *      *      *      *      *      *
TATAGTGAGTCTGATTAAATTTGATAAGCCAGTAAGCAGTGGGTCTCTAGTTAGCCAGAGAGCTCTGCT
ATATCACTCAGCATAATTAAGCTATTCCGGTCATTCTGTCACCCAAGAGATCAATCGGTCTCTCGAGACGA

      2600      2610      2620      2630      2640      2650      2660
      *      *      *      *      *      *      *
TATATAGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTT
ATATATCTGGAGGGTGGCATGTGCGGATGGCGGGTAAACGCAGTTACCCCGCCTCAACAATGCTGTAAAA

      2670      2680      2690      2700      2710      2720      2730
      *      *      *      *      *      *      *
GGAAAGTCCCGTTGATTTTGGTGCCAAAACAACTCCCATTTGACGTCAATGGGGTGGAGACTTGGAATC
CCTTTCAGGGCAACTAAAACCACGGTTTTGTTTGAGGGTAACTGCAGTTACCCACCTCTGAACCTTTAG

      2740      2750      2760      2770      2780      2790      2800
      *      *      *      *      *      *      *
CCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCACCATGGTAATAGCGAT
GGGCACTCAGTTTGGCGATAGGTGCGGGTAACTACATGACGGTTTTTGGCGTAGTGGTACCATTATCGCTA

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FIGURE 19 CONTINUED

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      2810      2820      2830      2840      2850      2860      2870
      *      *      *      *      *      *      *
GACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCACTGTACTGGGCATAATGCCAGGCG
CTGATTATGCATCTACATGACGGTTCATCCTTTCAGGGTATTCCAGTACATGACCCGTATTACGGTCCGC

      2880      2890      2900      2910      2920      2930      2940
      *      *      *      *      *      *      *
GGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGT
CCGGTAAATGGCAGTAAGTGCAGTTATCCCCCGCATGAACCGTATACTATGTGAACTACATGACGGTTCA

      2950      2960      2970      2980      2990      3000      3010
      *      *      *      *      *      *      *
GGCCAGTTTACCGTAAATAGTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAAC
CCCGTCAAATGGCATTTCAGGTGGTAAGTGCAGTTACCTTTCAGGGATAACCGCAATGATACCCCTTG

      3020      3030      3040      3050      3060      3070      3080
      *      *      *      *      *      *      *
ATACGTCATTATTGACGTCAATGGGCGGGGCGTCTGGGCGGTGAGCCAGGCGGGCCATTACCGTAAGT
TATGCAGTAATAACTGCAGTTACCCGCCCCCAGCAACCCGCCAGTCCGGTCCGCCCCGTAAATGGCATTCA

      3090      3100      3110      3120      3130      3140      3150
      *      *      *      *      *      *      *
TATGTAACGCGGAAGTCCATATATGGGCTATGAACTAATGACCCCGTAATTGATTACTATTAATAACTAG
ATACATTGCGCCTTGAGGTATATACCCGATACTTGATTACTGGGGCATTAACTAATGATAATTATTGATC

      3160      3170      3180      3190      3200      3210      3220
      *      *      *      *      *      *      *
TCAATAATCAATGTCTGCAATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCT
AGTTATTAGTTACAGGACGTAATTACTTAGCCGGTTGCGCGCCCCCTCTCCGCCAAACGCATAACCCGCGA

      3230      3240      3250      3260      3270      3280      3290
      *      *      *      *      *      *      *
CTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTCGGCTCGGCGAGCGGTATCAGCTCACTC
GAAGGCGAAGGAGCGAGTGAAGTGAAGGACCGGAGCCAGCAAGCCGACGCGCTCGCCATAGTCGAGTGAAG

      3300      3310      3320      3330      3340      3350      3360
      *      *      *      *      *      *      *
AAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAG
TTTCCGCCATTATGCCAATAGGTGTCTTAGTCCCTATTGCGTCTCTTCTTGTACACTCGTTTTCCGGTC

      3370      3380      3390      3400      3410      3420      3430
      *      *      *      *      *      *      *
CAAAAGGCCAGGAACCGTAAAAAGGCGCGCTTGGCTGGCGTTTTCATAGGCTCCGCCCCCTGACGAGC
GTTTTCCGGTCTTGGCATTTCCTCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGGACTGCTCG

      3440      3450      3460      3470      3480      3490      3500
      *      *      *      *      *      *      *
ATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAACGAGGCGTTTCC
TAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCTGATATTTCTATGGTCCGCAAAGG

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FIGURE 19 CONTINUED

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      3510      3520      3530      3540      3550      3560      3570
      *      *      *      *      *      *      *
CCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCGCTTACCGGATACCTGTCCGCTTTCTC
GGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGCGAATGGCCTATGGACAGGCGGAAAGAG

      3580      3590      3600      3610      3620      3630      3640
      *      *      *      *      *      *      *
CCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCT
GGAAGCCCTTCGCACCGCGAAAGAGTTACGAGTGGACATCCATAGAGTCAAGCCACATCCAGCAAGCGA

      3650      3660      3670      3680      3690      3700      3710
      *      *      *      *      *      *      *
CCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAAGTATCGTCT
GGTTCGACCCGACACACGTGCTTGGGGGGCAAGTGGGGCTGGCGACCGCGAATAGGCCATTGATAGCAGA

      3720      3730      3740      3750      3760      3770      3780
      *      *      *      *      *      *      *
TGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG
ACTCAGGTTGGGCCATTCTGTGCTGAATAGCGGTGACCGTCGTGGTGACCATTTGTCCTAATCGTCTCGC

      3790      3800      3810      3820      3830      3840      3850
      *      *      *      *      *      *      *
AGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAGGACAGTAT
TCCATACATCCGCCACGATGTCTCAAGAACTTCACCACCGGATTGATGCCGATGTGATCTTCTGTCTATA

      3860      3870      3880      3890      3900      3910      3920
      *      *      *      *      *      *      *
TTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAQA
AACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTCTCAACCATCGAGAACTAGGCCGTTTGT

      3930      3940      3950      3960      3970      3980      3990
      *      *      *      *      *      *      *
AACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAGCAGCAGATTACGGCGAGAAAAAAGGATCTCAA
TTGGTGGCGACCATCGCCACCAAAAAACAACGTTTCGTCTCTAATGCGCGTCTTTTTTCTAGAGTT

      4000      4010      4020      4030      4040      4050      4060
      *      *      *      *      *      *      *
GAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATTTTGG
CTTCTAGGAAACTAGAAAAGATGCCCCAGACTGCGAGTCACCTTGCTTTTGAGTGCAATTCCTTAAACC

      4070      4080      4090      4100      4110      4120      4130
      *      *      *      *      *      *      *
TCATGAACAATAAACTGTCTGCTTACATAAACAGTAATAACAAGGGGTGTTATGAGCCATATTCAACGGG
AGTACTTGTTATTTTGACAGACGAATGTATTTGTCATTATGTTCCCCACAATACTCGGTATAAGTTGCC

      4140      4150      4160      4170      4180      4190      4200
      *      *      *      *      *      *      *
AAACGTCCTTGCTCGAGGCCCGGATTAAATCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCG
TTTGAGAACGAGCTCCGGCGCTAATTTAAGGTTGTACCTACGACTAAATATACCCATATTTACCCGAGC

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FIGURE 19 CONTINUED

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      4210      4220      4230      4240      4250      4260      4270
      *      *      *      *      *      *      *
CGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTT
GCTATTACAGCCCGTTAGTCCACGCTGTTAGATAGCTAACATACCCTTCGGGCTACGCGGTCTCAACAAA

      4280      4290      4300      4310      4320      4330      4340
      *      *      *      *      *      *      *
CTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGG
GACTTTGTACCGTTTCCATCGCAACGGTTACTACAATGTCTACTCTACCAGTCTGATTTGACCGACTGCC

      4350      4360      4370      4380      4390      4400      4410
      *      *      *      *      *      *      *
AATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGCG
TTAAATACGGAGAAGGCTGGTAGTTCGTAAATAGGCATGAGGACTACTACGTACCAATGAGTGGTGACG

      4420      4430      4440      4450      4460      4470      4480
      *      *      *      *      *      *      *
GATCCCCGGGAAAACAGCATTCAGGTATTAGAAGATATCCTGATTCAGGTGAAAATATTGTTGATGCG
CTAGGGGCCCTTTTGTGCTAAGGTCCATAATCTTCTTATAGGACTAAGTCCACTTTTATAACAACTACGC

      4490      4500      4510      4520      4530      4540      4550
      *      *      *      *      *      *      *
CTGGCAGTGTTCCTGCGCCGTTGCATTGCTGTTTGTAAATTGTCTTTTAACAGCGATCGCGTAT
GACCGTCACAAGGACGCGGCCAACGTAAGCTAAGGACAAACATTACAGGAAAATTGTGCTAGCGCATA

      4560      4570      4580      4590      4600      4610      4620
      *      *      *      *      *      *      *
TTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCG
AAGCAGAGCGAGTCCGCGTTAGTGCTTACTTATTGCCAAACCACTACGCTCACTAAACTACTGCTCGC

      4630      4640      4650      4660      4670      4680      4690
      *      *      *      *      *      *      *
TAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCAGGATTTCAGTC
ATTACCGACCGGACAACTTGTTCAGACCTTTCTTTACGTATTTGAAAACGGTAAGAGTGGCCTAAGTCAG

      4700      4710      4720      4730      4740      4750      4760
      *      *      *      *      *      *      *
GTCACCTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATG
CAGTGAGTACCACTAAAGAGTGAACTATTGGAATAAAAAGTCTCCCCTTTAATTATCCAACATAACTAC

      4770      4780      4790      4800      4810      4820      4830
      *      *      *      *      *      *      *
TTGGACGAGTCGGAATCGCAGACCGATACCAAGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTTC
AACCTGCTCAGCCTTAGCGTCTGGCTATGGTCTAGAACGGTAGGATACCTTGACGGAGCCACTCAAAAG

      4840      4850      4860      4870      4880      4890      4900
      *      *      *      *      *      *      *
TCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTT
AGGAAGTAATGTCTTTGCCGAAAAGTTTTTATACCATAACTATTAGGACTATACTTATTTAACGTCAA

```

FIGURE 19 CONTINUED

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      4910      4920      4930      4940      4950      4960      4970
      *      *      *      *      *      *      *
CATTGATGCTCGATGAGTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATCATGA
GTAAACTACGAGCTACTCAAAAAGATTAGTCTTAACCAATTAACCAACATTGTGACCGTCTCGTAGTACT

      4980      4990      5000      5010      5020      5030      5040
      *      *      *      *      *      *      *
GCGGATACATATTTGAATGTATTTAGAAAAATAAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGT
CGCCTATGTATAAACTTACATAAATCTTTTTATTTGTTTATCCCAAGGCGCGTGTAAGGGGCTTTTCA

      5050
      *      *
GCCACCTGACGTC
CGGTGGACTGCAG
```

FIGURE 19 CONTINUED

```

      10      20      30      40      50      60      70
      *      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGGAATGCAGGTGCAGATCCAGAGCCTGTTTCTGCTCCTCCTGTGGGTGCCCCG
CGATCGCGCGCGGTGGTACCCCTTACGTCCACGTCTAGGTCTCGGACAAAGACGAGGAGGACACCCACGGGC
      M G M Q V Q I Q S L F L L L L W V P>

      80      90      100      110      120      130      140
      *      *      *      *      *      *      *
GGTCCAGAGGACACACCCCTGTGGAAGGCCGGAATCCTGTATAAGGCCAAGTTCGTGGCTGCCTGGACCCT
CCAGGTCTCCTGTGTGGGACACCTTCCGGCCCTTAGGACATATTCCGGTTCAAGCACCGACGGACCTGGGA
G S R G H T L W K A G I L Y K A K F V A A W T L>

      150      160      170      180      190      200      210
      *      *      *      *      *      *      *
GAAGGCTGCCGCTTTCTGCTTAGCGATTTCTTTCTAGCGTGAAGCTGACCCCACTGTGCGTGACCCCTG
CTTCCGACGGCGAAAGGACGGATCGCTAAAGAAAGGATCGCACTTCGACTGGGGTGACACGCACTGGGAC
K A A A F L P S D F F P S V K L T P L C V T L>

      220      230      240      250      260      270      280
      *      *      *      *      *      *      *
TATATGGATGACGTGGTGTGGGAGCCAGCATCATCACTTCGAGAAGCTGGGACTGTCCAGATACGTGG
ATATACCTACTGCAACCACGACCCCTCGGTCTAGTAGTGAAGCTCTTCGACCCCTGACAGGTCTATGCACC
Y M D D V V I G A S I I N F E K L G L S R Y V>

      290      300      310      320      330      340      350
      *      *      *      *      *      *      *
CTAGGCTGATCCTGAAGGAGCCTGTGCACGGCGTGTCCACCCTGCCAGAGACCACCGTGGTGAGGAGGAC
GATCCGACTAGGACTTCCTCGGACACGTGCCGACAGGTGGGACGGTCTCTGGTGGCACCCTCCTCCTG
A R L I L K E P V H G V S T L P E T T V V R R T>

      360      370      380      390      400      410
      *      *      *      *      *      *
CGTGTACTATGGAGTGCCTGTGTGGAAGTGGCTGAGCCTGCTGGTGGCCCTTTGTGGGTACC
GCACATGATACCTCACGGACACACCTTCACCGACTCGGACGACCACGGGAAACACCCATGG
V Y Y G V P V W K W L S L L V F F V G T>

```

FIGURE 20

```

10      20      30      40      50      60      70
*      *      *      *      *      *      *
GCTAGCGCCGCCACCATGGGAATGCAGGTGCAGATCCAGAGCCTGTTTCTGCTCCTCCTGTGGGTGCCCCG
CGATCGCGGCGGTGGTACCCTTACGTCCACGTCTAGGTCTCGGACAAAGACGAGGAGGACACCCACGGGC
      M G M Q V Q I Q S L F L L L L W V P>

      80      90      100      110      120      130      140
*      *      *      *      *      *      *
GGTCCAGAGGACACACCCTGTGGAAGGCCGGAATCCTGTATAAGGCCAAGTTCGTGGCTGCCTGGACCCT
CCAGGTCTCCTGTGTGGGACACCTTCCGGCCCTTAGGACATATCCGGTTCAAGCACCGACGGACCTGGGA
G S R G H T L W K A G I L Y K A K F V A A W T L>

      150      160      170      180      190      200      210
*      *      *      *      *      *      *
GAAGGCTGCCGCTTTCCTGCCTAGCGATTTCTTTCTAGCGTGAAGCTGACCCCACTGTGCGTGACCCTG
CTTCCGACGGCGAAAGGACGGATCGCTAAAGAAAGGATCGCACTTCGACTGGGGTGACACGCACTGGGAC
K A A A F L P S D F F P S V K L T P L C V T L>

      220      230      240      250      260      270      280
*      *      *      *      *      *      *
TATATGGATGACGTGGTGGTGGGAGTGGGACTGTCCAGGTACGTGGCTAGGCTGATCCTGAAGGAGCCTG
ATATACCTACTGCACCACGACCCTCACCCTGACAGGTCCATGCACCGATCCGACTAGGACTTCCTCGGAC
Y M D D V V L G V G L S R Y V A R L I L K E P>

      290      300      310      320      330      340      350
*      *      *      *      *      *      *
TGCACGGCGTGTCCACCCTGCCAGAGACCACCGTGGTGAGGAGGACCGTGTACTATGGAGTGCCTGTGTG
ACGTGCCGCACAGGTGGGACGGTCTCTGGTGGCACCCTCCTCCTGGCACATGATACCTCACGGACACAC
V H G V S T L P E T T V V R R T V Y Y G V P V W>

      360      370      380      390
*      *      *      *
GAAGTGGCTGAGCCTGCTGGTGGCCCTTTGTGTGAGGTACC
CTTCACCGACTCGGACGACCACGGGAAACACACTCCATGG
K W L S L L V P F V *>

```

FIGURE 21

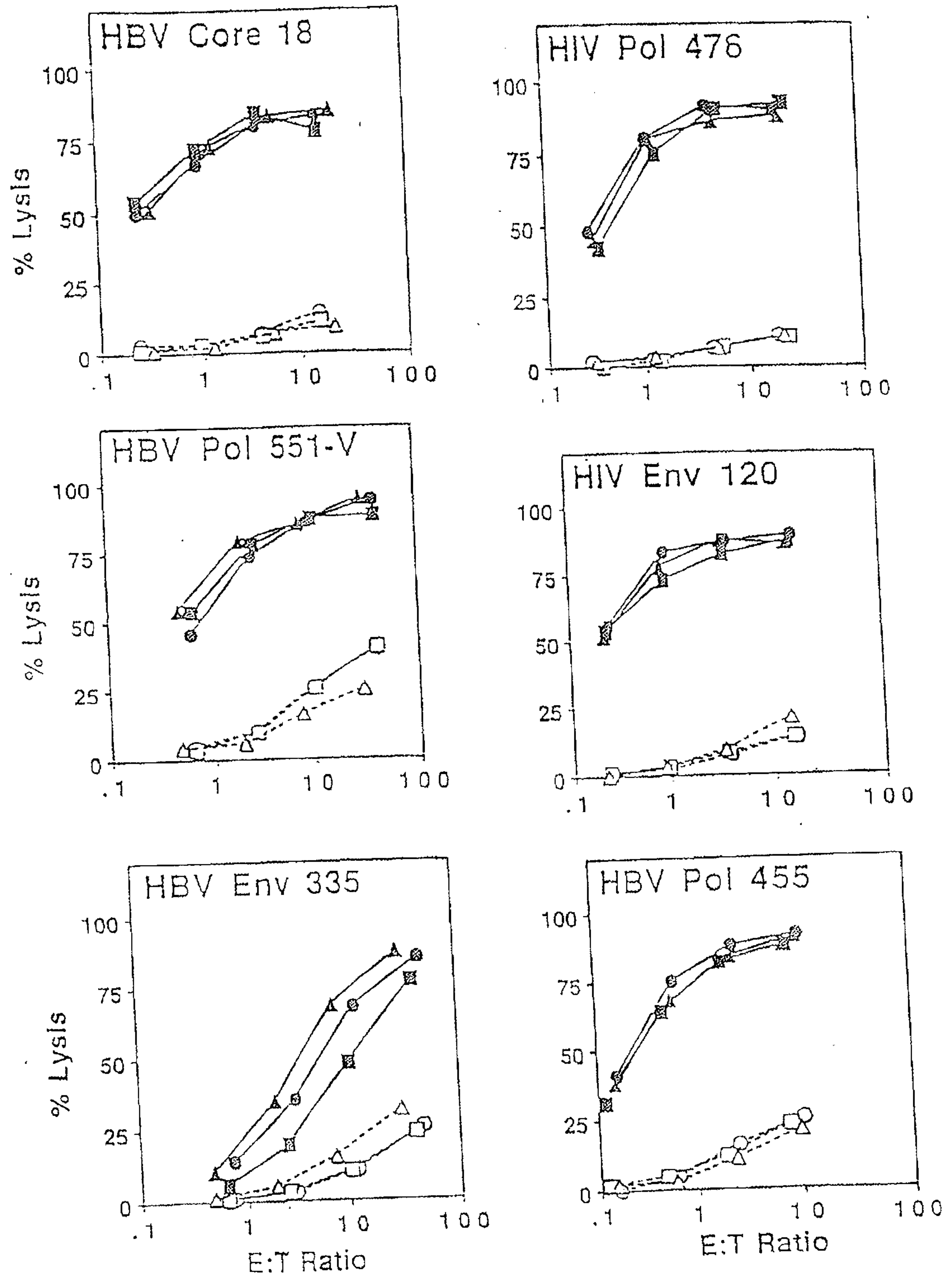


FIGURE 22

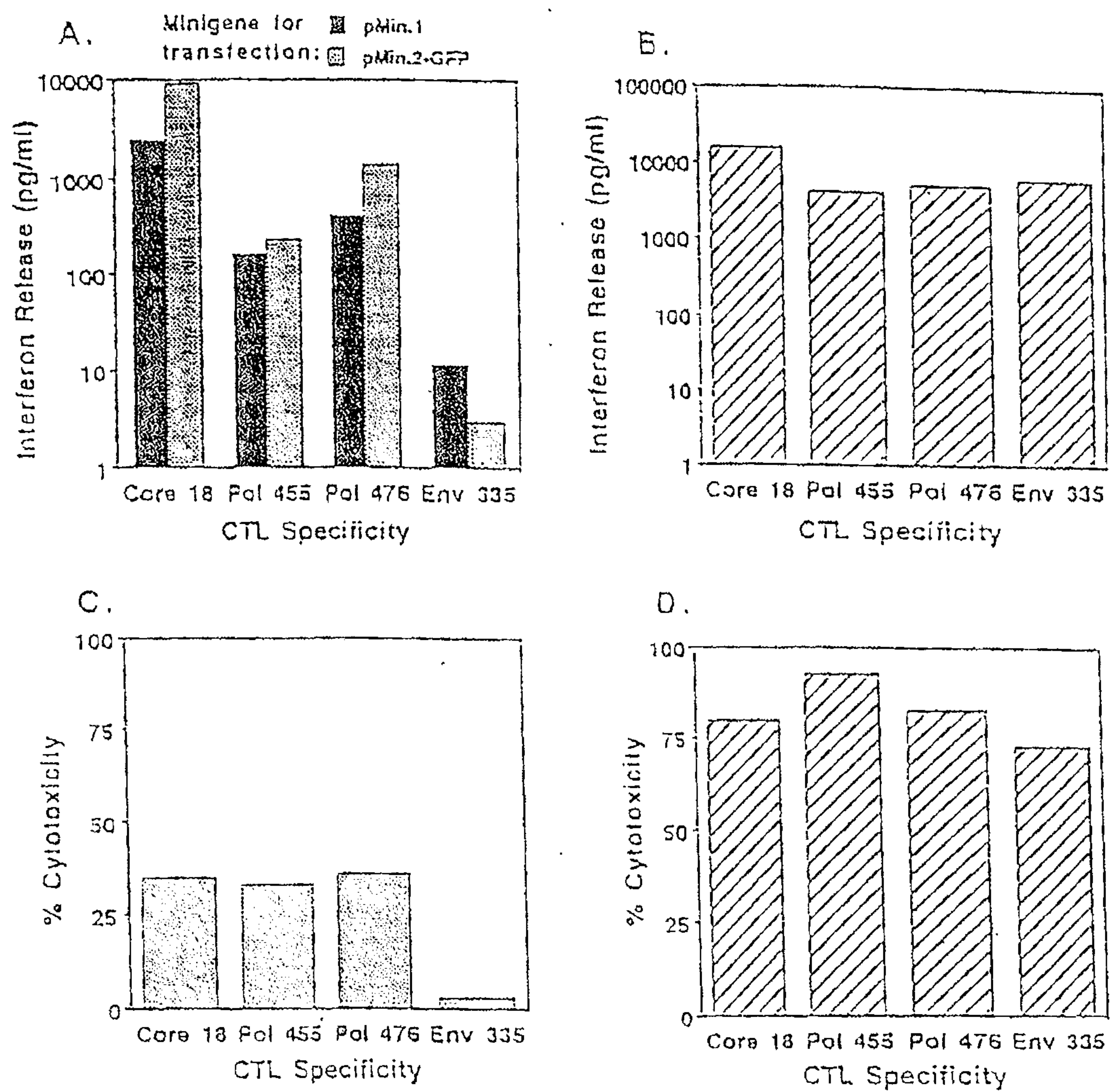


FIGURE 23

A. pMin.1-No PADRE

PADRE deleted										
	↓									
sig seq	HBV Pol 149	HBV Core 18	HIV Env 120	HBV Pol 551-V	HBV Pol 455	HIV Pol 476	HBV Core 141	HIV Env 49	HBV Env 335	

B. pMin.1-Anchor

Pol 538 native anchor (A at P9)										
	↓									
sig seq	HBV Pol 149	PADRE	HBV Core 18	HIV Env 120	HBV Pol 551-A	HBV Pol 455	HIV Pol 476	HBV Core 141	HIV Env 49	HBV Env 335

C. pMin.1-No Sig

Signal sequence deleted										
	↓									
	HBV Pol 149	PADRE	HBV Core 18	HIV Env 120	HBV Pol 551-V	HBV Pol 455	HIV Pol 476	HBV Core 141	HIV Env 49	HBV Env 335

D. pMin.1-Switch

Position of HBV Env 335 and HBV Pol 455 switched										
	↓									
sig seq	HBV Pol 149	PADRE	HBV Core 18	HIV Env 120	HBV Pol 551-V	HBV Env 335	HIV Pol 476	HBV Core 141	HIV Env 49	HBV Pol 455

FIGURE 24

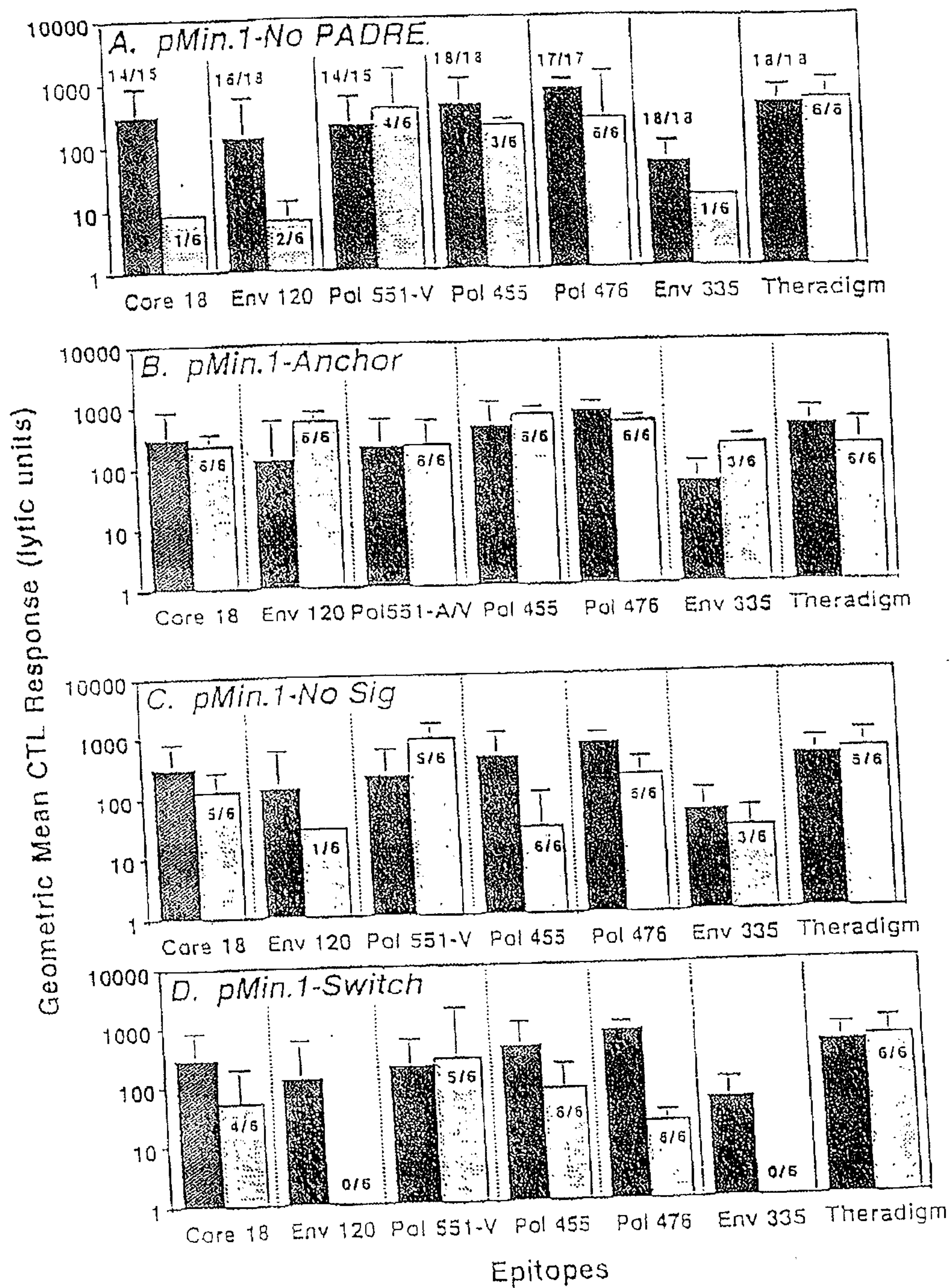


FIGURE 25