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(71) Applicant(s)
The Trustees of the University of Pennsylvania

(72) Inventor(s)
WEINER, David B;YAN, Jian;LADDY, Dominick

(74) Agent / Attorney
FB Rice Pty Ltd, Level 29 126 Phillip Street, Sydney, NSW, 2000, AU

ABSTRACT

Improved anti-HIV immunogens and nucleic acid molecules that encode them are disclosed. Immunogens disclosed include those having consensus sequences for HIV Subtype A Envelope protein, those having consensus sequences for HIV Subtype B Envelope protein, those having consensus sequences for HIV Subtype C Envelope protein, those having consensus sequences for HIV Subtype D Envelope protein, those having consensus sequences for HIV Subtype B consensus Nef-Rev protein, and those having consensus sequences form HIV Gag protein subtypes A, B, C and D. Improved anti-HPV immunogens and nucleic acid molecules that encode them; improved anti-HCV immunogens and nucleic acid molecules that encode them; improved hTERT immunogens and nucleic acid molecules that encode them; and improved anti-Influenza immunogens and nucleic acid molecules that encode them are disclosed. Pharmaceutical composition, recombinant vaccines comprising and live attenuated pathogens are disclosed as well methods of inducing an immune response in an individual against HIV, HPV, HCV, hTERT and Influenza are disclosed.

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COMPLETE SPECIFICATION
STANDARD PATENT

Invention Title:

Improved vaccines and methods for using the same

The following statement is a full description of this invention including the best method of performing it known to us:

IMPROVED VACCINES AND METHODS FOR USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional application pursuant to Section 79B of the *Patents Act 1990* of Australian Patent Application No. 2024200014, which is a divisional application of Australian Patent No. 2019229397, which is a divisional application of Australian Patent No. 2017208322, which is a divisional application of Australian Patent No. 2015234338, which is a divisional application of Australian Patent No. 2013203229, which is a divisional application of Australian Patent No. 2013201592, which is a divisional application of Australian Patent No. 2007278831 which corresponds to International Application No. PCT/US2007/074769 filed 30 July 2007 in the Australian national phase, which claims priority to USSN 60/833,856 filed 28 July 2006, USSN 60/833,861 filed 28 July 2006 and USSN 60/890,352 filed 2 February 2007, the complete contents of which are incorporated herein in their entirety.

FIELD OF THE INVENTION

The present invention relates to improved HIV, HPV, HCV, Influenza and cancer vaccines, improved methods for inducing immune responses, and for prophylactically and/or therapeutically immunizing individuals against HIV, HPV, HCV, Influenza and cancer.

BACKGROUND OF THE INVENTION

The HIV genome is highly plastic due to a high mutation rate and functional compensation. This high mutation rate is driven by at least two mechanisms: the low fidelity of the viral reverse transcriptase (RT) resulting in at least one mutation per replication cycle, and the dual effects of the anti-retroviral cellular factor APOBEC3G gene and viral infectivity factor Vif accessory gene. Genomes with every possible mutation and many double mutations are generated during every replication cycle, resulting in tremendous antigenic diversity. Accordingly, it has been argued that a candidate vaccine derived from an individual isolate may not elicit sufficient cross reactivity to protect against diverse circulating HIV viruses. Recent studies have suggested that consensus immunogens (Gao, F., et al. 2005. Antigenicity and immunogenicity of a synthetic human immunodeficiency virus type 1 group m consensus

envelope glycoprotein. J Virol 79:1154-63.; Scriba, T. J., et al. 2005. Functionally-inactive and immunogenic Tat, Rev and Nef DNA vaccines derived from sub-Saharan subtype C human immunodeficiency virus type 1 consensus sequences. Vaccine 23:1158-69) or ancestral immunogens (Doria-Rose, N. A., et al. 2005. Human Immunodeficiency Virus Type 1 subtype B Ancestral Envelope Protein Is Functional and Elicits Neutralizing Antibodies in Rabbits Similar to Those Elicited by a Circulating Subtype B Envelope. J. Virol. 79:11214-11224; Gao, F., et al. 2004. Centralized immunogens as a vaccine strategy to overcome HIV-1 diversity. Expert Rev. Vaccines 3:S161-S168; Mullins, J. I., et al. 2004. Immunogen sequence: the fourth tier of AIDS vaccine design. Expert Rev. Vaccines 3:S151-S159; Nickle, D. C., et al. 2003. Consensus and ancestral state HIV vaccines. Science 299:1515-1517) may be useful in this regard. However, the initial studies of these approaches showed relatively modest cellular immune enhancement induced by these immunogens.

Recently Derdeyn et al. analyzed HIV-1 subtype C envelope glycoprotein sequences in eight African heterosexual transmission pairs and found that shorter V1, V2 and V4 length and fewer glycans are the common features shared by the sequences obtained from early transmitters (Derdeyn, C. A., et al. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 303:2019-2022.). This data suggests that antigens that mimic such viruses might have relevance for the early-transmitted viruses. However, such early transmitter structures have not been observed for all subtypes (Chohan, B., et al. 2005. Selection for Human Immunodeficiency Virus Type 1 envelope glycosylation variants with shorter V1-V2 loop sequences occurs during transmission of certain genetic subtypes and may impact viral RNA levels. J. Virol. 79:6528-6531). However, incorporation of shorter V loops in an envelope immunogen may have other benefits, such as enhancement of sensitivity to soluble CD4 (Pickora, C., et al. 2005. Identification of two N-linked glycosylation sites within the core of the Simian Immunodificiency virus glycoprotein whose removal enhances sensitivity to soluble CD4. J. Virol. 79:12575-12583), and should be considered.

Studies have shown the importance of HIV-1 specific CTL responses in controlling viral load during acute and asymptomatic infection and the development of AIDS. However, it is unclear if current envelope based DNA vaccines are as potent as needed. Several methods have been used to increase the expression levels of HIV-1 immunogens, such as codon optimization

(Andre, S., et al. 1998. Increased immune response elicited by DNA vaccination with a synthetic gp120 sequence with optimized codon usage. J Virol 72:1497-503; Deml, L., et al. A. 2001. Multiple effects of codon usage optimization on expression and immunogenicity of DNA candidate vaccines encoding the human immunodeficiency virus type 1 gag protein. J. Virol. 75:10991-11001), RNA optimization (Muthumani, K., et al. 2003. Novel engineered HIV-1 East African Clade-A gp160 plasmid construct induces strong humoral and cell-mediated immune responses in vivo. Virology 314:134-46; Schneider, R., M. et al. 1997. Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows Rev-independent expression of Gag and Gag/protease and particle formation. J. Virol. 71:4892-4903) and the addition of immunoglobin leader sequences that have weak RNA secondary structure (Yang, J. S., et al.. 2001. Induction of potent Th1-Type immune responses from a novel DNA vaccine for West Nile Virus New York Isolate (WNV-NY1999). J. Infect Diseases 184:809-816).

Human Papillomavirus (HPV) has a circular dsDNA genome (7,000–8,000 base pairs). There are up to 200 different genotypes. Phylogenetically, HPV is highly conserved. Mucosal HPV are Classified as “High Risk” or “Low Risk”. The Low Risk group includes types 6, 11, 42, and others. Associated Diseases include: Genital Warts; Low grade cervical, anal, vulvar, vaginal dysplasia; and Recurrent Respiratory Papillomatosis. The High Risk group includes types 16, 18, 31, 33, 45, 52, 58, and others. Associated Diseases include: Essential cause of Cervical cancer, pre-cancerous dysplasia; major cause of Anal, vulvar, vaginal, tonsillar cancer; and co-factor for other aerodigestive cancer. Every Day, 800 women die of cervical cancer.

HPV E6 and E7 proteins are tumor-specific antigens, required for tumorigenesis and maintenance of the tumor state. E7-specific immune responses are deleted in cervical cancer patients. Both E6 and E7 proteins interact specifically with the products of cellular human tumor suppressor genes, E6 with p53 and E7 with Rb (retinoblastoma tumor suppressor gene). E6 and E7 are ideal immunotherapeutic targets.

hTERT is a human telomerase reverse transcriptase that synthesizes a TTAGGG tag on the end of telomeres to prevent cell death due to chromosomal shortening. Embryonic cells and some germ line cells normally express hTERT to regulate homeostasis of cell populations. Cancer cells, however, exploit this mechanism of regulation to disrupt homeostasis of cell

populations. For instance, hTERT over-expression occurs in more than 85% of human cancer cells. Therefore, hTERT is an ideal immunotherapeutic target.

hTERT may also enhance immunotherapeutics against hyperproliferating cells expressing hTERT due to HCV or HPV infection. The E6 oncoprotein from high-risk HPV types activates human telomerase reverse transcriptase (hTERT) transcription in human keratinocytes. Dysplastic legions and early neoplastic legions within the liver also express hTERT at abnormally high levels. Thus, immunotherapy against HPV and HCV may be enhanced by targeting cells that express hTERT at abnormal levels. Combination immunotherapy using both hTERT and HPV or HCV proteins or nucleic acids encoding such proteins is an attractive immunotherapy.

Influenza Hemagglutinin (HA) is expressed on the surface of Influenza viral particles and is responsible for initial contact between the virus and its host cell. HA is a well-known immunogen. Influenza A strain H1N5, an avian influenza strain, particularly threatens the human population because of its HA protein which, if slightly genetically reassorted by natural mutation, has greatly increased infectivity of human cells as compared to other strains of the virus. Infection of infants and older or immunocompromised adults humans with the viral H1N5 strain is often correlated to poor clinical outcome. Therefore, HA and other influenza molecules of the H1N5 strain of Influenza are ideal immunotherapeutic targets.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

SUMMARY OF THE INVENTION

The present invention relates to nucleic acid constructs and proteins encoded thereby which provide improved immunogenic targets against which an anti-HIV immune response can be generated.

The present invention provides consensus sequences for HIV Subtype A Envelope protein, consensus sequences for HIV Subtype B Envelope protein, consensus sequences for HIV Subtype C Envelope protein, consensus sequences for HIV Subtype D Envelope protein,

consensus sequences for HIV Subtype B consensus Nef-Rev protein, and consensus sequences form HIV Gag protein subtypes A, B, C and D.

The present invention provides constructs which encode such proteins sequences, vaccines which comprise such proteins and/or nucleic acid molecules that encode such proteins, and methods of inducing anti-HIV immune responses.

The present invention relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; fragments of SEQ ID NO:1; sequences having at least 90% homology to SEQ ID NO:1; fragments of sequences having at least 90% homology to SEQ ID NO:1; SEQ ID NO:3; fragments of SEQ ID NO:3; sequences having at least 90% homology to SEQ ID NO:3; fragments of sequences having at least 90% homology to SEQ ID NO:3; SEQ ID NO:5; fragments of SEQ ID NO:5; sequences having at least 90% homology to SEQ ID NO:5; fragments of sequences having at least 90% homology to SEQ ID NO:5; SEQ ID NO:7; fragments of SEQ ID NO:7; sequences having at least 90% homology to SEQ ID NO:7; fragments of sequences having at least 90% homology to SEQ ID NO:7; SEQ ID NO:9; fragments of SEQ ID NO:9; sequences having at least 90% homology to SEQ ID NO:9; fragments of sequences having at least 90% homology to SEQ ID NO:9; SEQ ID NO:11; fragments of SEQ ID NO:11; sequences having at least 90% homology to SEQ ID NO:11; fragments of sequences having at least 90% homology to SEQ ID NO:11.

The present invention relates to nucleic acid molecule that encode a protein selected from the group consisting of: SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21.

The present invention relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:2; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:2; fragments of nucleotide sequences that encode SEQ ID NO:2; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:2; nucleotide sequences that encode SEQ ID NO:4; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:4; fragments of nucleotide sequences that encodes SEQ ID NO:4; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:4; nucleotide sequences that encode

SEQ ID NO:6; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:6; fragments of nucleotide sequences that encode SEQ ID NO:6; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:6; nucleotide sequences that encode SEQ ID NO:8; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:8; fragments of nucleotide sequences that encodes SEQ ID NO:8; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:8; nucleotide sequences that encode SEQ ID NO:10; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:10; fragments of nucleotide sequences that encode SEQ ID NO:10; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:10; nucleotide sequences that encode SEQ ID NO:12; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:12; fragments of nucleotide sequences that encodes SEQ ID NO:12; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:12.

The present invention further provides pharmaceutical compositions comprising such nucleic acid molecules and their use in methods of inducing an immune response in an individual against HIV that comprise administering to an individual a composition comprising such nucleic acid molecules.

The present invention further provides recombinant vaccine comprising such nucleic acid molecules and their use in methods of inducing an immune response in an individual against HIV that comprise administering to an individual such a recombinant vaccine.

The present invention further provides live attenuated pathogens comprising such nucleic acid molecules and their use in methods of inducing an immune response in an individual against HIV that comprise administering to an individual such live attenuated pathogens.
live attenuated pathogen

The present invention further provides proteins comprising amino acid sequences selected from the group consisting of: SEQ ID NO:2, sequences having at least 90% homology to SEQ ID NO:2; fragments of SEQ ID NO:2; fragments of sequences having at least 90% homology to SEQ ID NO:2; SEQ ID NO:4, sequences having at least 90% homology to SEQ ID

NO:4; fragments of SEQ ID NO:; fragments of sequences having at least 90% homology to SEQ ID NO:4; SEQ ID NO:6, sequences having at least 90% homology to SEQ ID NO:6; fragments of SEQ ID NO:6; fragments of sequences having at least 90% homology to SEQ ID NO:6; SEQ ID NO:8, sequences having at least 90% homology to SEQ ID NO:8; fragments of SEQ ID NO:8; fragments of sequences having at least 90% homology to SEQ ID NO:8; SEQ ID NO:10, sequences having at least 90% homology to SEQ ID NO:10; fragments of SEQ ID NO:10; fragments of sequences having at least 90% homology to SEQ ID NO:10; SEQ ID NO:12, sequences having at least 90% homology to SEQ ID NO:12; fragments of SEQ ID NO:12; and fragments of sequences having at least 90% homology to SEQ ID NO:12.

The present invention further provides proteins comprising amino acid sequences selected from the group consisting of: SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21.

The present invention further provides pharmaceutical compositions comprising such proteins and their use in methods of inducing an immune response in an individual against HIV that comprise administering to an individual a composition comprising such proteins.

The present invention further provides recombinant vaccine comprising such proteins and their use in methods of inducing an immune response in an individual against HIV that comprise administering to an individual such a recombinant vaccine.

The present invention further provides live attenuated pathogens comprising such proteins and their use in methods of inducing an immune response in an individual against HIV that comprise administering to an individual such live attenuated pathogens.

Proteins comprising consensus HPV genotype 16 E6-E7 amino acid sequences and nucleic acid molecules that comprising a nucleotide sequence encoding such proteins are provided.

The present invention relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:22; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:22; and fragments thereof.

The present invention also relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: a nucleic acid sequence that encodes SEQ ID NO:23; a nucleic acid sequence that encodes SEQ ID NO:24; a nucleic acid sequence that

encodes SEQ ID NO:25; a nucleic acid sequence that encodes SEQ ID NO:26; and a nucleic acid sequence that encodes SEQ ID NO:27.

The present invention also relates to pharmaceutical composition such nucleic acid molecules and to methods of inducing an immune response in an individual against HPV comprising administering to said individual a composition comprising such nucleic acid molecules.

The present invention further relates to recombinant vaccines comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HPV comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogen comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HPV comprising administering to said individual such live attenuated pathogens.

The present invention also relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: proteins comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:23, fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:23; and fragments thereof.

The present invention also relates to proteins comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; and SEQ ID NO:27.

The present invention also relates to pharmaceutical compositions comprising such proteins and to methods of inducing an immune response in an individual against HPV comprising administering to said individual a composition comprising such proteins.

The present invention also relates to recombinant vaccines comprising such proteins and to method of inducing an immune response in an individual against HPV comprising administering to said individual such recombinant vaccines.

The present invention also relates to live attenuated pathogens comprising such protein and to methods of inducing an immune response in an individual against HPV comprising administering to said individual such live attenuated pathogens.

Proteins comprising consensus HCV genotype 1a and 1b E1-E2 amino acid sequences and nucleic acid molecules that comprising a nucleotide sequence encoding such proteins are provided.

The present invention relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:30; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:30; and fragments thereof.

The present invention also relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: a nucleic acid sequence that encodes SEQ ID NO:31.

The present invention also relates to pharmaceutical composition such nucleic acid molecules and to methods of inducing an immune response in an individual against HCV comprising administering to said individual a composition comprising such nucleic acid molecules.

The present invention further relates to recombinant vaccines comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HCV comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogen comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HCV comprising administering to said individual such live attenuated pathogens.

The present invention also relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: proteins comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:31; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:31; and fragments thereof.

The present invention also relates to pharmaceutical compositions comprising such proteins and to methods of inducing an immune response in an individual against HCV comprising administering to said individual a composition comprising such proteins.

The present invention also relates to recombinant vaccines comprising such proteins and to method of inducing an immune response in an individual against HCV comprising administering to said individual such recombinant vaccines.

The present invention also relates to live attenuated pathogens comprising such protein and to methods of inducing an immune response in an individual against HCV comprising administering to said individual such live attenuated pathogens.

Proteins comprising consensus hTERTamino acid sequences and nucleic acid molecules that comprising a nucleotide sequence encoding such proteins are provided.

The present invention further relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO: 34; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO: 34; and fragments thereof.

The present invention also relates to pharmaceutical composition such nucleic acid molecules and to methods of inducing an immune response in an individual against hyperproliferative cells expressing hTERT comprising administering to said individual a composition comprising such nucleic acid molecules.

The present invention further relates to recombinant vaccines comprising such nucleic acid molecules and methods of inducing an immune response in an individual against hyperproliferative cells expressing hTERT comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogen comprising such nucleic acid molecules and methods of inducing an immune response in an individual against hyperproliferative cells expressing hTERT comprising administering to said individual such live attenuated pathogens.

The present invention also relates to nucleic acid molecules that comprising a nucleotide sequence selected from the group consisting of: proteins comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:35; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:35; and fragments thereof.

The present invention also relates to pharmaceutical compositions comprising such proteins and to methods of inducing an immune response in an individual against hyperproliferative cells expressing hTERT comprising administering to said individual a composition comprising such proteins.

The present invention also relates to recombinant vaccines comprising such proteins and to method of inducing an immune response in an individual against hyperproliferative cells expressing hTERT comprising administering to said individual such recombinant vaccines.

The present invention also relates to live attenuated pathogens comprising such protein and to methods of inducing an immune response in an individual against hyperproliferative cells expressing hTERT comprising administering to said individual such live attenuated pathogens.

Proteins comprising Influenza H5N1 consensus HA amino acid sequences, Influenza H1N1 and H5N1 consensus NA amino acid sequences, Influenza H1N1 and H5N1 consensus M1 amino acid sequences, and Influenza H5N1 consensus M2E-NP amino acid sequences and nucleic acid molecules that comprising a nucleotide sequence encoding such proteins are provided.

The present invention further relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:36; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:36; and fragments thereof.

The present invention further relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:38; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:38; and fragments thereof.

The present invention further relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:40; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:40; and fragments thereof.

The present invention further relates to nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:42; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:42; and fragments thereof.

The present invention also relates to pharmaceutical compositions comprising such nucleic acid molecules and to methods of inducing an immune response in an individual against HPV, HCV, and Influenza virus comprising administering to said individual a composition comprising such nucleic acid molecules.

The present invention further relates to recombinant vaccines comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HPV,

HCV, and Influenza virus comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogens comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HPV, HCV, and Influenza virus comprising administering to said individual such live attenuated pathogens.

The present invention also relates to pharmaceutical compositions comprising such nucleic acid molecules and to methods of inducing an immune response in an individual against HPV, HCV, and Influenza virus comprising administering to said individual a composition comprising such nucleic acid molecules.

The present invention further relates to recombinant vaccines comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HPV, HCV, and Influenza virus comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogens comprising such nucleic acid molecules and methods of inducing an immune response in an individual against HPV, HCV, and Influenza virus comprising administering to said individual such live attenuated pathogens.

The present invention further relates to protein molecules comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:37; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:37; and fragments thereof.

The present invention further relates to protein molecules comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:39; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:39; and fragments thereof.

The present invention further relates to protein molecules comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:41; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:41; and fragments thereof.

The present invention further relates to protein molecules comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:43; fragments thereof; nucleotide sequences having at least 90% homology to SEQ ID NO:43; and fragments thereof.

The present invention also relates to pharmaceutical compositions comprising such protein molecules and to methods of inducing an immune response in an individual against Influenza virus comprising administering to said individual a composition comprising such protein molecules.

The present invention further relates to recombinant vaccines comprising such protein molecules and methods of inducing an immune response in an individual against Influenza virus comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogens comprising such protein molecules and methods of inducing an immune response in an individual against Influenza virus comprising administering to said individual such live attenuated pathogens.

The present invention also relates to pharmaceutical compositions comprising such protein molecules and to methods of inducing an immune response in an individual against Influenza virus comprising administering to said individual a composition comprising such protein molecules.

The present invention further relates to recombinant vaccines comprising such protein molecules and methods of inducing an immune response in an individual against Influenza virus comprising administering to said individual such a recombinant vaccine.

The present invention further relates to live attenuated pathogens comprising such protein molecules and methods of inducing an immune response in an individual against Influenza virus comprising administering to said individual such live attenuated pathogens.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a comparison of the amino acid sequences of EY2E1-B and EK2P-B. The IgE leader sequence is underlined. The boxed regions show variable regions. The * denotes six important residues involved in CCR5 utilization. The cleavage site is indicated by an arrow. The transmembrane domain is shown by the dotted line.

Figure 2 shows phylogenetic relationships of two HIV-1 subtype B envelope sequences. Forty-two HIV-1 subtype B envelope sequences, EY2E1-B, EK2P-B, two subtype D and two subtype C sequences (outgroup) were included in the phylogenetic analysis. The subtype B envelope sequences representing a broad sample of diversity were from the following 11

countries: Argentinia (1); Australia (6); China (1); France (4); Germany (1); Great Britain (2); Italy (1); Japan (1); The Netherlands (4); Spain (1); United States (20). The EY2E1-B and EK2P-B sequences are shown in black boxes.

Figure 3 shows expression of envelope immunogens. Panel A shows results from Western blotting analysis of EY2E1-B and EK2P-B genes. RD cells were transfected with different plasmids. 48 hours later, cell lysates were collected. Samples were analyzed by Western blotting and probed with HIV-1 gp120 monoclonal (2G12). As for loading control, the blot was stripped and reprobed with a monoclonal anti-actin antibody. Panel B shows results from immunofluorescence assay of EY2E1-B and EK2P-B genes. The transfected RD cells expressing envelope proteins showed typical red fluorescence. HIV-1 envelope-specific monoclonal antibody F105 served as the source of primary antibody.

Figure 4. shows total IgG antibody titers in the sera of the immunized mice. Panel A shows the measurement of subtype B envelope-specific antibody responses. Panel B shows the measurement of subtype A/E envelope-specific antibody responses. Panel C shows the measurement of subtype C envelope-specific antibody responses. Humoral immune responses after immunization with DNA constructs pEY2E1-B and pEK2P-B were detected by enzyme-linked immunosorbent assay (ELISA). Each mouse was immunized intramuscularly with three times, each of 100 μg of DNA at bi-weekly intervals. Mice from each group (n=3) were bled one week after the third immunization and equally pooled sera were diluted in blocking buffer and analyzed as described in Materials and Methods. Pooled sera collected from mice immunized with pVAX were used as a control. Absorbance (OD) was measured at 450 nm. Each data point represents averaged three OD values from three mice sera per group and values represent the mean of ELISA obtained in three separate assays.

Figure 5 shows induction of cell-mediated immune responses by pEY2E1-B in both BalB/C mice and HLA-A2 transgenic mice. Frequencies of subtype B consensus envelope-specific IFN-γ spot forming cells (SFC) per million splenocytes after DNA vaccination with pEY2E1-B and pEK2P-B were determined by ELISpot assay in both BalB/C mice (Panel A) and transgenic mice (Panel C). Frequencies of CD8 depleted, subtype B consensus envelope-specific IFN-γ spot forming cells per million splenocytes after DNA vaccination with pEY2E1-B and pEK2P-B were also determined in both BalB/C mice (Panel B) and transgenic mice (Panel D).

The slenocytes were isolated from individual immunized mice (three mice per group) and stimulated in vitro with overlapping consensus subtype B envelope peptides pools. Backbone pVAX immunized mice were included as a negative control. The values are the means + standard deviations of the means of IFN- γ SFCs. (Panel E) Characterization of subtype B consensus envelope-specific dominant epitopes. The splenocytes collected from pEY2E1-B and pEK2P-B vaccinated BalB/C mice, respectively, were cultured with 29 HIV-1 subtype B consensus envelope peptide pools for 24 hours. IFN- γ secreting cells were determined by ELISpot assay as described above.

Figure 6 shows cross reactivity induced by pEY2E1-B in both BalB/C mice and HLA-A2 transgenic mice. The additive T-cell immune responses in BalB/C mice induced by vaccination with pEY2E1-B and pEK2P-B against four individual peptide pools of HIV-1 MN envelope peptides (Panel A), HIV-1 group M (Panel B), subtype C consensus envelope peptides (Panel C) and two subtype C isolate envelope peptides (Panels D and E) were measured by IFN-γ ELISpot assay. The additive T-cell immune responses in HLA-A2 transgenic mice induced by vaccination with pEY2E1-B and pEK2P-B against four individual peptide pools of HIV-1 MN envelope peptides (Panel F), HIV-1 group M (Panel G), subtype C consensus envelope peptides (Panel H) and two subtype C isolate envelope peptides (Panels I and J) were also measured. Backbone pVAX immunized mice were included as a negative control.

Figure 7 show characterization of subtype B MN envelope-specific dominant epitopes in both BalB/C mice (Panel A) and HLA-A2 transgenic mice (Panel B) immunized with pEY2E1-B and pEK2P-B. The splenocytes collected from pEY2E1-B and pEK2P-B vaccinated BalB/C mice and transgenic mice, respectively, were cultured with 29 HIV-1 subtype B MN envelope peptide pools for 24 hours. IFN-γ secreting cells were determined by ELISpot assay as described above.

Figure 8 shows a schematic representation of functional domains of E72E1-B (about 700+ amino acids).

Figure 9 shows a map of E72E1-B construct.

Figure 10 Panels A and B, show that a strong cellular immune response is induced E72E1-B.

Figure 11 Panels A and B, show that strong and broad cross-reactive cellular immune responses are induced E72E1-B.

Figure 12 Panels A-D show that strong cross-clade cellular immune responses are induced E72E1-B.

Figure 13 depicts the immunogen designed for study in Example 2.

Figure 14 shows phylogenetic relationships: Thirty-Six HIV-1 subtype C envelope sequences, EY3E1-C, EK3P-C, two subtype B, one subtype A and one subtype D sequences (outgroup) were included in the phylogenetic analysis. The subtype C envelope sequences representing a broad sample of diversity were from 12 countries.

Figure 15 Panels A and B show data from studies of cellular response elicited by pEY3E1-C.

Figure 16 shows data from studies of cellular responses elicited by pEY3E1-C.

Figure 17 Panels A-D show data from studies of cross-reactive cellular responses elicited by pEY3E1-C within the same clade.

Figure 18 Panels A and B show data from studies of cross-reactive cellular responses elicited by pEY3E1-C. Panel A shows data from subtype C (Uruguay) env-Specific IFN-γ ELISpot. Panel B shows data from Subtype C (S. Africa) env-Specific IFN-γ ELISpot.

Figure 19 Panels A-F show data from studies of cross-reactive cellular responses elicited by pEY3E1-C between clades.

Figure 20 Panels A-X show data from studies of immune responses elicited by HIV-1 gag consensus constructs.

Figure 21 illustrates the HPV life cycle in the genital tract epithelium.

Figure 22 shows a map of HPV-16 genome organization.

Figure 23 illustrates immunogen design: * refers to deletions or mutations important for p53 binding and degradation; Δ refers to mutations in Rb binding site.

Figure 24 includes an illustration of the genetic construct p1667 which includes coding sequences for HPV E6 and E7 proteins, and pVAX, the backpbone plasmid which lacks the HPV insert and is used a negative control.

Figure 25 Panels A-D show cellular immune responses induced by the DNA immunogen p1667.

Figure 26 shows results of immunodominant epitope mapping.

Figure 27 shows results from the prophylactic experiments using E6/E7 DNA Vaccine to study protection in C57/BL6 Mice.

Figure 28 shows results from the tumor regression experiments using E6/E7 DNA Vaccine to study protection in C57/BL6 Mice.

Figure 29 shows the data from experiments detecting E7 Tetramer positive lymphocytes in spleens.

Figure 30 shows the data from experiments detecting E7 Tetramer positive lymphocytes in tumors.

Figure 31 shows data from a DNA Vaccine protection study in transgenic mice.

Figure 32 shows enhanced cellular immune responses to HIV-1 consensus immunogens with IM co-injection of plasmid encoded IL-12 followed by electroporation (EP). IFNγ ELISpots were performed two weeks after the (**a**) first immunization, (**b**) second immunization, and (**c**) third immunization (as seen in comparison to the other three). Responses to env are depicted as black bars and gag are depicted as white bars with the data shown as stacked group mean responses ± SEM.

Figure 33 shows enhanced cross-reactive cellular immune responses with intramuscular electroporation. After three immunizations, the total T-cell immune response in pEY2E1-B immunized macaques against four peptide pools of the HIV-1 group M peptides were determined by IFNγ ELISpot. The data are shown as stacked group means ± SEM.

Figure 34 shows Enhanced memory responses to HIV-1 immunogens with IM electroporation and plasmid IL-12. Five months after the last immunization, ELISpot assays were performed to determine antigen-specific memory responses to gag and env in the IM and EP immunized groups with and without co-immunization with the IL-12 plasmid. The data are shown as group mean responses ± SEM.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or

group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein, the phrase "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a nucleic acid molecule will hybridize another a nucleic acid molecule, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C. lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present in excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C. for short probes, primers or oligonucleotides (e.g. 10 to 50 nucleotides) and at least about 60°C. for longer probes, primers or oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Sequence homology for nucleotides and amino acids may be determined using FASTA, BLAST and Gapped BLAST (Altschul et al., Nuc. Acids Res., 1997, 25, 3389, which is incorporated herein by reference in its entirety) and PAUP* 4.0b10 software (D. L. Swofford, Sinauer Associates, Massachusetts). “Percentage of similarity” is calculated using PAUP* 4.0b10 software (D. L. Swofford, Sinauer Associates, Massachusetts). The average similarity of the consensus sequence is calculated compared to all sequences in the phylogenic tree (see Figures 2 and 14).

Briefly, the BLAST algorithm, which stands for Basic Local Alignment Search Tool is suitable for determining sequence similarity (Altschul et al., J. Mol. Biol., 1990, 215, 403-410, which is incorporated herein by reference in its entirety). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence

pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension for the word hits in each direction are halted when: 1) the cumulative alignment score falls off by the quantity X from its maximum achieved value; 2) the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or 3) the end of either sequence is reached. The Blast algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The Blast program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff et al., Proc. Natl. Acad. Sci. USA, 1992, 89, 10915-10919, which is incorporated herein by reference in its entirety) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands. The BLAST algorithm (Karlin et al., Proc. Natl. Acad. Sci. USA, 1993, 90, 5873-5787, which is incorporated herein by reference in its entirety) and Gapped BLAST perform a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide sequences would occur by chance. For example, a nucleic acid is considered similar to another if the smallest sum probability in comparison of the test nucleic acid to the other nucleic acid is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

As used herein, the term “genetic construct" refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered.

As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

Overview

The present invention provides improved vaccines by utilizing a multi-phase strategy to enhance cellular immune responses induced by immunogens. Modified consensus sequences for immunogens were generated. Genetic modifications including codon optimization, RNA optimization, and the addition of a high efficient immunoglobin leader sequence to increase the immunogenicity of constructs are also disclosed. The novel immunogens have been designed to elicit stronger and broader cellular immune responses than a corresponding codon optimized immunogens.

The invention provides improved HIV, HPV, HCV, Influenza and cancer vaccines by providing proteins and genetic constructs that encode proteins with epitopes that make them particularly effective as immunogens against which anti-HIV, anti-HPV, anti-HCV, antri-influenze and anti-hTert immune responses, respectively, can be induced. Accordingly, vaccines can be provided to induce a therapeutic or prophylactic immune response. In some embodiments, the means to deliver the immunogen is a DNA vaccine, a recombinant vaccine, a protein subunit vaccine, a composition comprising the immunogen, an attenuated vaccine or a killed vaccine. In some embodiments, the vaccine comprises a combination selected from the groups consisting of: one or more DNA vaccines, one or more recombinant vaccines, one or more protein subunit vaccines, one or more compositions comprising the immunogen, one or more attenuated vaccines and one or more killed vaccines.

According to some embodiments of the invention, a vaccine according to the invention is delivered to an individual to modulate the activity of the individual's immune system and thereby enhance the immune response against HIV, HPV, HCV, Influenza or hTERT. When a nucleic acid molecules that encodes the protein is taken up by cells of the individual the nucleotide sequence is expressed in the cells and the protein are thereby delivered to the individual. Aspects of the invention provide methods of delivering the coding sequences of the protein on nucleic acid molecule such as plasmid, as part of recombinant vaccines and as part of attenuated vaccines, as isolated proteins or proteins part of a vector.

According to some aspects of the present invention, compositions and methods are provided which prophylactically and/or therapeutically immunize an individual against HIV, HIV, HPV, HCV, Influenza and cancer.

The present invention relates to compositions for delivering nucleic acid molecules that comprise a nucleotide sequence that encodes a protein of the invention operably linked to regulatory elements. Aspects of the present invention relate to compositions a recombinant vaccine comprising a nucleotide sequence that encodes that encodes a protein of the invention; a live attenuated pathogen that encodes a protein of the invention and/or includes a protein of the invention; a killed pathogen includes a protein of the invention; or a composition such as a liposome or subunit vaccine that comprises a protein of the invention. The present invention further relates to injectable pharmaceutical compositions that comprise compositions.

HIV

The present invention provides improved anti-HIV vaccines by utilizing a multi-phase strategy to enhance cellular immune responses induced by HIV immunogens. Modified consensus sequences for immunogens were generated Genetic modifications including codon optimization, RNA optimization, and the addition of a high efficient immunoglobin leader sequence to increase the immunogenicity of constructs are also disclosed. The novel immunogens have been designed to elicit stronger and broader cellular immune responses than a corresponding codon optimized immunogens.

SEQ ID NO:1 is a subtype A consensus envelope DNA sequence construct. SEQ ID NO:1 comprises coding sequence for HIV vaccine sequence that comprises an IgE leader sequence linked to a consensus sequence for Subtype A envelope protein. SEQ ID NO:2 comprises the amino acid sequence for HIV vaccine sequence construct that comprises an IgE leader sequence linked to a consensus sequence for Subtype A envelope protein. The IgE leader sequence is SEQ ID NO:15. SEQ ID NO:16 is the Subtype A consensus Envelope protein sequence.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:16, fragment thereof, a nucleic acid molecule that encodes SEQ ID NO:16, or fragments thereof. In some embodiments, vaccines of the invention preferably include SEQ ID NO:2 or a nucleic acid molecule that encodes it. In some embodiments, vaccines of the invention preferably include SEQ ID NO:1. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:15 or nucleic acid sequence which encodes the same.

Fragments of SEQ ID NO:1 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:1 may comprise 180 or more nucleotides; in some embodiments, 270 or more nucleotides; in some embodiments 360 or more nucleotides; in some embodiments, 450 or more nucleotides; in some embodiments 540 or more nucleotides; in some embodiments, 630 or more nucleotides; in some embodiments, 720 or more nucleotides; in some embodiments, 810 or more nucleotides; in some embodiments, 900 or more nucleotides; in some embodiments, 990 or more nucleotides; in some embodiments, 1080 or more nucleotides; in some embodiments, 1170 or more nucleotides; in some embodiments, 1260 or more nucleotides; in some embodiments, 1350 or more nucleotides in some embodiments, 1440 or more nucleotides; in some embodiments, 1530 or more nucleotides; in some embodiments, 1620 or more nucleotides; in some embodiments, 1710 or more nucleotides; in some embodiments, 1800 or more nucleotides; in some embodiments, 1890 or more nucleotides; in some embodiments, 1980 or more nucleotides; and in some embodiments, 2070 or more nucleotides. In some embodiments, fragments of SEQ ID NO:1 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:1 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 180 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 450 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 630 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 810 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 990 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1170 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1350 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1530 nucleotides, in some embodiments fewer than 1620 nucleotides, in some embodiments fewer than 1710 nucleotides, in some embodiments fewer than 1800 nucleotides, in some embodiments fewer than 1890 nucleotides, in some embodiments fewer than 1980 nucleotides, in some embodiments fewer than 1020 nucleotides, and in some embodiments fewer than 2070 nucleotides.

Fragments of SEQ ID NO:2 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:2 may comprise 60 or more amino acids; in some embodiments, 90 or more amino acids; in some embodiments, 120 or more amino acids; in some embodiments; 150 or more amino acids; in some embodiments 180 or more amino acids; in some embodiments, 210 or more amino acids; in some embodiments, 240 or more amino acids; in some embodiments, 270 or more amino acids; in some embodiments, 300 or more amino acids; in some embodiments, 330 or more amino acids; in some embodiments, 360 or more amino acids; in some embodiments, 390 or more amino acids; in some embodiments, 420 or more amino acids; in some embodiments, 450 or more amino acids; in some embodiments, 480 or more amino acids; in some embodiments, 510 or more amino acids; in some embodiments, 540 or more amino acids; in some embodiments, 570 or more amino acids; in some embodiments, 600 or more amino acids; in some embodiments, 630 or more amino acids; in some embodiments, 660 or more amino acid; and in some embodiments, 690 or more amino acids. Fragments may comprise fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 330 amino acids, in some embodiments fewer than 360 amino acids, in some embodiments fewer than 390 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 450 amino acids, in some embodiments fewer than 480 amino acids, in some embodiments fewer than 540 amino acids, in some embodiments fewer than 600 amino acids, in some embodiments fewer than 660 amino acids, and in some embodiments fewer than 690 amino acids.

SEQ ID NO:3 is a subtype B consensus envelope DNA sequence construct. SEQ ID NO:3 comprises coding sequence for HIV vaccine sequence that comprises an IgE leader sequence linked to a consensus sequence for Subtype B envelope protein. SEQ ID NO:4 comprises the amino acid sequence for HIV vaccine sequence construct that comprises an IgE leader sequence linked to a consensus sequence for Subtype B envelope protein. The IgE leader sequence is SEQ ID NO:15. SEQ ID NO:17 is the Subtype B consensus Envelope protein sequence.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:17, fragment thereof, a nucleic acid molecule that encodes SEQ ID NO:17, or fragments thereof. In some embodiments, vaccines of the invention preferably include SEQ ID NO:4 or a nucleic acid molecule that encodes it. In some embodiments, vaccines of the invention preferably include SEQ ID NO:3. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:15 or nucleic acid sequence which encodes the same.

Fragments of SEQ ID NO:3 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:3 may comprise 180 or more nucleotides; in some embodiments, 270 or more nucleotides; in some embodiments 360 or more nucleotides; in some embodiments, 450 or more nucleotides; in some embodiments 540 or more nucleotides; in some embodiments, 630 or more nucleotides; in some embodiments, 720 or more nucleotides; in some embodiments, 810 or more nucleotides; in some embodiments, 900 or more nucleotides; in some embodiments, 990 or more nucleotides; in some embodiments, 1080 or more nucleotides; in some embodiments, 1170 or more nucleotides; in some embodiments, 1260 or more nucleotides; in some embodiments, 1350 or more nucleotides in some embodiments, 1440 or more nucleotides; in some embodiments, 1530 or more nucleotides; in some embodiments, 1620 or more nucleotides; in some embodiments, 1710 or more nucleotides; in some embodiments, 1800 or more nucleotides; in some embodiments, 1890 or more nucleotides; in some embodiments, 1980 or more nucleotides; in some embodiments, 2070 or more nucleotides; in some embodiments, 2160 or more nucleotides; in some embodiments, 2250 or more nucleotides; in some embodiments, 2340 or more nucleotides; in some embodiments, 2430 or more nucleotides; in some embodiments, 2520 or more nucleotides; in some embodiments, 2620 or more nucleotides; and in some embodiments, 2700 or more nucleotides. In some embodiments, fragments of SEQ ID NO:3 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:3 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 180 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 450 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 630 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 810 nucleotides, in some embodiments fewer than

900 nucleotides, in some embodiments fewer than 990 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1170 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1350 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1530 nucleotides, in some embodiments fewer than 1620 nucleotides, in some embodiments fewer than 1710 nucleotides, in some embodiments fewer than 1800 nucleotides, in some embodiments fewer than 1890 nucleotides, in some embodiments fewer than 1980 nucleotides, in some embodiments fewer than 1020 nucleotides, in some embodiments fewer than 2070 nucleotides, in some embodiments fewer than 2160 nucleotides, in some embodiments fewer than 2250 nucleotides, in some embodiments fewer than 2340 nucleotides, in some embodiments fewer than 2430 nucleotides, in some embodiments fewer than 2520 nucleotides, in some embodiments fewer than 2610 nucleotides, and in some embodiments fewer than 2700 nucleotides.

Fragments of SEQ ID NO:4 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:4 may comprise 60 or more amino acids; in some embodiments, 90 or more amino acids; in some embodiments, 120 or more amino acids; in some embodiments; 150 or more amino acids; in some embodiments 180 or more amino acids; in some embodiments, 210 or more amino acids; in some embodiments, 240 or more amino acids; in some embodiments, 270 or more amino acids; in some embodiments, 300 or more amino acids; in some embodiments, 330 or more amino acids; in some embodiments, 360 or more amino acids; in some embodiments, 390 or more amino acids; in some embodiments, 420 or more amino acids; in some embodiments, 450 or more amino acids; in some embodiments, 480 or more amino acids; in some embodiments, 510 or more amino acids; in some embodiments, 540 or more amino acids; in some embodiments, 570 or more amino acids; in some embodiments, 600 or more amino acids; in some embodiments, 630 or more amino acids; in some embodiments, 660 or more amino acid; and in some embodiments, 690 or more amino acids. Fragments may comprise fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 330 amino acids, in

some embodiments fewer than 360 amino acids, in some embodiments fewer than 390 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 450 amino acids, in some embodiments fewer than 480 amino acids, in some embodiments fewer than 540 amino acids, in some embodiments fewer than 600 amino acids, in some embodiments fewer than 660 amino acids, and in some embodiments fewer than 690 amino acids.

SEQ ID NO:5 is a subtype C consensus envelope DNA sequence construct. SEQ ID NO:5 comprises coding sequence for HIV vaccine sequence that comprises an IgE leader sequence linked to a consensus sequence for Subtype C envelope protein. SEQ ID NO:6 comprises the amino acid sequence for HIV vaccine sequence construct that comprises an IgE leader sequence linked to a consensus sequence for Subtype C envelope protein. The IgE leader sequence is SEQ ID NO:15. SEQ ID NO:18 is the Subtype C consensus Envelope protein sequence.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:18, fragment thereof, a nucleic acid molecule that encodes SEQ ID NO:18, or fragments thereof. In some embodiments, vaccines of the invention preferably include SEQ ID NO:6 or a nucleic acid molecule that encodes it. In some embodiments, vaccines of the invention preferably include SEQ ID NO:5. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:15 or nucleic acid sequence which encodes the same.

Fragments of SEQ ID NO:5 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:5 may comprise 180 or more nucleotides; in some embodiments, 270 or more nucleotides; in some embodiments 360 or more nucleotides; in some embodiments, 450 or more nucleotides; in some embodiments 540 or more nucleotides; in some embodiments, 630 or more nucleotides; in some embodiments, 720 or more nucleotides; in some embodiments, 810 or more nucleotides; in some embodiments, 900 or more nucleotides; in some embodiments, 990 or more nucleotides; in some embodiments, 1080 or more nucleotides; in some embodiments, 1170 or more nucleotides; in some embodiments, 1260 or more nucleotides; in some embodiments, 1350 or more nucleotides in some embodiments, 1440 or more nucleotides; in some embodiments, 1530 or more nucleotides; in some embodiments, 1620 or more nucleotides; in some embodiments, 1710 or more nucleotides; in some embodiments, 1800 or more nucleotides; in some embodiments, 1890 or more nucleotides; in some embodiments,

1980 or more nucleotides; and in some embodiments, 2070 or more nucleotides. In some embodiments, fragments of SEQ ID NO:5 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:5 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 180 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 450 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 630 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 810 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 990 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1170 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1350 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1530 nucleotides, in some embodiments fewer than 1620 nucleotides, in some embodiments fewer than 1710 nucleotides, in some embodiments fewer than 1800 nucleotides, in some embodiments fewer than 1890 nucleotides, in some embodiments fewer than 1980 nucleotides, in some embodiments fewer than 1020 nucleotides, and in some embodiments fewer than 2070 nucleotides.

Fragments of SEQ ID NO:6 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:6 may comprise 60 or more amino acids; in some embodiments, 90 or more amino acids; in some embodiments, 120 or more amino acids; in some embodiments; 150 or more amino acids; in some embodiments 180 or more amino acids; in some embodiments, 210 or more amino acids; in some embodiments, 240 or more amino acids; in some embodiments, 270 or more amino acids; in some embodiments, 300 or more amino acids; in some embodiments, 330 or more amino acids; in some embodiments, 360 or more amino acids; in some embodiments, 390 or more amino acids; in some embodiments, 420 or more amino acids; in some embodiments, 450 or more amino acids; in some embodiments, 480 or more amino acids; in some embodiments, 510 or more amino acids; in some embodiments, 540 or more amino acids; in some embodiments, 570 or more amino acids; in some embodiments, 600 or more amino acids; in some embodiments, 630 or more amino acids; in some embodiments, 660 or more amino acid; and in some embodiments, 690 or more amino

acids. Fragments may comprise fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 330 amino acids, in some embodiments fewer than 360 amino acids, in some embodiments fewer than 390 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 450 amino acids, in some embodiments fewer than 480 amino acids, in some embodiments fewer than 540 amino acids, in some embodiments fewer than 600 amino acids, in some embodiments fewer than 660 amino acids, and in some embodiments fewer than 690 amino acids.

SEQ ID NO:7 is a subtype D consensus envelope DNA sequence construct. SEQ ID NO:7 comprises coding sequence for HIV vaccine sequence that comprises an IgE leader sequence linked to a consensus sequence for Subtype D envelope protein. SEQ ID NO:8 comprises the amino acid sequence for HIV vaccine sequence construct that comprises an IgE leader sequence linked to a consensus sequence for Subtype D envelope protein. The IgE leader sequence is SEQ ID NO:15. SEQ ID NO:19 is the Subtype D consensus Envelope protein sequence.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:19, fragment thereof, a nucleic acid molecule that encodes SEQ ID NO:19, or fragments thereof. In some embodiments, vaccines of the invention preferably include SEQ ID NO:8 or a nucleic acid molecule that encodes it. In some embodiments, vaccines of the invention preferably include SEQ ID NO:7. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:15 or nucleic acid sequence which encodes the same.

Fragments of SEQ ID NO:7 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:7 may comprise 180 or more nucleotides; in some embodiments, 270 or more nucleotides; in some embodiments 360 or more nucleotides; in some embodiments, 450 or more nucleotides; in some embodiments 540 or more nucleotides; in some embodiments, 630 or more nucleotides; in some embodiments, 720 or more nucleotides; in some embodiments, 810 or more nucleotides; in some embodiments, 900 or more nucleotides; in some embodiments, 990 or more nucleotides; in some embodiments, 1080 or more nucleotides; in

some embodiments, 1170 or more nucleotides; in some embodiments, 1260 or more nucleotides; in some embodiments, 1350 or more nucleotides in some embodiments, 1440 or more nucleotides; in some embodiments, 1530 or more nucleotides; in some embodiments, 1620 or more nucleotides; in some embodiments, 1710 or more nucleotides; in some embodiments, 1800 or more nucleotides; in some embodiments, 1890 or more nucleotides; in some embodiments, 1980 or more nucleotides; and in some embodiments, 2070 or more nucleotides; and in some embodiments, 2140 or more nucleotides. In some embodiments, fragments of SEQ ID NO:7 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:7 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 180 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 450 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 630 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 810 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 990 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1170 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1350 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1530 nucleotides, in some embodiments fewer than 1620 nucleotides, in some embodiments fewer than 1710 nucleotides, in some embodiments fewer than 1800 nucleotides, in some embodiments fewer than 1890 nucleotides, in some embodiments fewer than 1980 nucleotides, in some embodiments fewer than 1020 nucleotides, in some embodiments fewer than 2070 nucleotides and in some embodiments fewer than 2140 nucleotides.

Fragments of SEQ ID NO:8 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:8 may comprise 60 or more amino acids; in some embodiments, 90 or more amino acids; in some embodiments, 120 or more amino acids; in some embodiments; 150 or more amino acids; in some embodiments 180 or more amino acids; in some embodiments, 210 or more amino acids; in some embodiments, 240 or more amino acids; in some embodiments, 270 or more amino acids; in some embodiments, 300 or more amino acids; in some embodiments, 330 or more amino acids; in some embodiments, 360 or more

amino acids; in some embodiments, 390 or more amino acids; in some embodiments, 420 or more amino acids; in some embodiments, 450 or more amino acids; in some embodiments, 480 or more amino acids; in some embodiments, 510 or more amino acids; in some embodiments, 540 or more amino acids; in some embodiments, 570 or more amino acids; in some embodiments, 600 or more amino acids; in some embodiments, 630 or more amino acids; in some embodiments, 660 or more amino acid; and in some embodiments, 690 or more amino acids. Fragments may comprise fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 330 amino acids, in some embodiments fewer than 360 amino acids, in some embodiments fewer than 390 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 450 amino acids, in some embodiments fewer than 480 amino acids, in some embodiments fewer than 540 amino acids, in some embodiments fewer than 600 amino acids, in some embodiments fewer than 660 amino acids, and in some embodiments fewer than 690 amino acids.

SEQ ID NO:9 is a subtype B Nef-Rev consensus envelope DNA sequence construct. SEQ ID NO:9 comprises coding sequence for HIV vaccine sequence that comprises an IgE leader sequence linked to a consensus sequence for Subtype B Nef-Rev protein. SEQ ID NO:10 comprises the amino acid sequence for HIV vaccine sequence construct that comprises an IgE leader sequence linked to a consensus sequence for Subtype B Nef-Rev protein. The IgE leader sequence is SEQ ID NO:15. SEQ ID NO:20 is the Subtype B Nef-Rev consensus protein sequence.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:20 fragment thereof, a nucleic acid molecule that encodes SEQ ID NO:20, or fragments thereof. In some embodiments, vaccines of the invention preferably include SEQ ID NO:10 or a nucleic acid molecule that encodes it. In some embodiments, vaccines of the invention preferably include SEQ ID NO:9. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:15 or nucleic acid sequence which encodes the same.

Fragments of SEQ ID NO:9 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:9 may comprise 180 or more nucleotides; in some embodiments, 270 or more nucleotides; in some embodiments 360 or more nucleotides; in some embodiments, 450 or more nucleotides; in some embodiments 540 or more nucleotides; in some embodiments, 630 or more nucleotides; in some embodiments, 720 or more nucleotides; in some embodiments, 810 or more nucleotides; in some embodiments, 900 or more nucleotides; and in some embodiments, 990 or more nucleotides; in some embodiments. In some embodiments, fragments of SEQ ID NO:9 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:9 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 180 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 450 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 630 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 810 nucleotides, in some embodiments fewer than 900 nucleotides, and in some embodiments fewer than 990 nucleotides.

Fragments of SEQ ID NO:2 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:2 may comprise 60 or more amino acids; in some embodiments, 90 or more amino acids; in some embodiments, 120 or more amino acids; in some embodiments; 150 or more amino acids; in some embodiments 180 or more amino acids; in some embodiments, 210 or more amino acids; in some embodiments, 240 or more amino acids; in some embodiments, 270 or more amino acids; in some embodiments, 300 or more amino acids; and in some embodiments, 330 or more amino acids.

SEQ ID NO:11 is a Gag consensus DNA sequence of subtype A, B, C and D DNA sequence construct. SEQ ID NO:11 comprises coding sequence for HIV vaccine sequence that comprises an IgE leader sequence linked to a consensus sequence for Gag consensus subtype A, B, C and D protein. SEQ ID NO:12 comprises the amino acid sequence for HIV vaccine sequence construct that comprises an IgE leader sequence linked to a consensus sequence for Gag subtype A, B, C and D protein. The IgE leader sequence is SEQ ID NO:15. SEQ ID NO:21 is the consensus Gag subtype A, B, C and D protein sequence.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:21, fragment thereof, a nucleic acid molecule that encodes SEQ ID NO:21, or fragments thereof. In some embodiments, vaccines of the invention preferably include SEQ ID NO:12 or a nucleic acid molecule that encodes it. In some embodiments, vaccines of the invention preferably include SEQ ID NO:11. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:15 or nucleic acid sequence which encodes the same.

Fragments of SEQ ID NO:11 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:11 may comprise 180 or more nucleotides; in some embodiments, 270 or more nucleotides; in some embodiments 360 or more nucleotides; in some embodiments, 450 or more nucleotides; in some embodiments 540 or more nucleotides; in some embodiments, 630 or more nucleotides; in some embodiments, 720 or more nucleotides; in some embodiments, 810 or more nucleotides; in some embodiments, 900 or more nucleotides; in some embodiments, 990 or more nucleotides; in some embodiments, 1080 or more nucleotides; in some embodiments, 1170 or more nucleotides; in some embodiments, 1260 or more nucleotides; in some embodiments, 1350 or more nucleotides in some embodiments, 1440 or more nucleotides; in some embodiments, 1530 or more nucleotides; in some embodiments, 1620 or more nucleotides; in some embodiments, 1710 or more nucleotides; and in some embodiments, 1800 or more nucleotides. In some embodiments, fragments of SEQ ID NO:11 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:11 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 180 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 450 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 630 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 810 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 990 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1170 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1350 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1530 nucleotides, in some embodiments fewer than 1620

nucleotides, in some embodiments fewer than 1710 nucleotides, and in some embodiments fewer than 1800 nucleotides.

Fragments of SEQ ID NO:12 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:12 may comprise 60 or more amino acids; in some embodiments, 90 or more amino acids; in some embodiments, 120 or more amino acids; in some embodiments; 150 or more amino acids; in some embodiments 180 or more amino acids; in some embodiments, 210 or more amino acids; in some embodiments, 240 or more amino acids; in some embodiments, 270 or more amino acids; in some embodiments, 300 or more amino acids; in some embodiments, 330 or more amino acids; in some embodiments, 360 or more amino acids; in some embodiments, 390 or more amino acids; in some embodiments, 420 or more amino acids; in some embodiments, 450 or more amino acids; in some embodiments, 480 or more amino acids; and in some embodiments, 510 or more amino acids. Fragments may comprise fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 330 amino acids, in some embodiments fewer than 360 amino acids, in some embodiments fewer than 390 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 450 amino acids, in some embodiments fewer than 480 amino acids, and in some embodiments fewer than 510 amino acids.

HPV

SEQ ID NO:22 comprises coding sequence for HPV vaccine sequence that comprises and IgE leader sequence, a consensus sequence for HPV E6, linked to a consensus sequence for HPV E7 by a proteolytic cleavage sequence. The consensus sequence for HPV E6 includes the immunodominant epitope set forth in SEQ ID NO:24. The consensus sequence for HPV E7 includes the immunodominant epitope set forth in SEQ ID NO:25. The consensus sequence for HPV E6 is SEQ ID NO:26. The consensus sequence for HPV E6 is SEQ ID NO:27. The IgE leader sequence is SEQ ID NO:28. A proteolytic cleavage sequence useful to link the two consensus sequences is SEQ ID NO:29.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:24 and/or SEQ ID NO:25, or nucleic acid sequence which encode one of both of them. Vaccines of the invention preferably include SEQ ID NO:27 and/or the SEQ ID NO:28, or nucleic acid sequences which encode one or both of them. Vaccines of the invention preferably include SEQ ID NO:27 linked to SEQ ID NO:28 by a proteolytic cleavage sequence such as SEQ ID NO:29, or nucleic acid sequence which encodes the fusion protein. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:28 or nucleic acid sequence which encodes the same. Vaccines of the invention preferably include SEQ ID NO:23 or the nucleic acid sequence in SEQ ID NO:22.

In some embodiments, proteins comprises fragments of SEQ ID NO:23. In some embodiments, proteins consist of fragments of SEQ ID NO:23. In some embodiments, nucleic acids comprises fragment of SEQ ID NO:22. In some embodiments, nucleic acids consist of a fragment of SEQ ID NO:22.

Fragments of SEQ ID NO:22 may comprise 30 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 45 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 60 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 75 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 90 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 120 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 150 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 180 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 210 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 240 or more nucleotides, including preferably sequences that

encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 270 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 300 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 360 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 420 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 480 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 540 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 600 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 300 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 660 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 720 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise 780 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:22 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:22 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 60 nucleotides, in some embodiments fewer than 75 nucleotides, in some embodiments fewer than 90 nucleotides, in some embodiments fewer than 120 nucleotides, in some embodiments fewer than 150 nucleotides, in some embodiments fewer than 180 nucleotides, in some embodiments fewer than 210 nucleotides, in some embodiments fewer than 240 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 300 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 420 nucleotides, in some embodiments fewer than 480 nucleotides, in some

embodiments fewer than 540 nucleotides, in some embodiments fewer than 600 nucleotides, in some embodiments fewer than 660 nucleotides, in some embodiments fewer than 720 nucleotides, and in some embodiments fewer than 780 nucleotides.

Fragments of SEQ ID NO:23 may comprise 15 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 18 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 21 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 24 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 30 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 36 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 42 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 48 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 54 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 60 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 18 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 72 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 90 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 120 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 150 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments,

fragments of SEQ ID NO:23 may comprise 180 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 210 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 240 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise 260 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:23 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:23 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 24 amino acids, in some embodiments fewer than 30 amino acids, in some embodiments fewer than 36 amino acids, in some embodiments fewer than 42 amino acids, in some embodiments fewer than 48 amino acids, in some embodiments fewer than 54 amino acids, in some embodiments fewer than 60 amino acids, in some embodiments fewer than 72 amino acids, in some embodiments fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids in some embodiments fewer than 240 amino acids, and in some embodiments fewer than 260 amino acids.

HCV

SEQ ID NO:30 comprises coding sequence for HCV vaccine sequence that comprises and IgE leader sequence, a consensus sequence for HCV E1, linked to a consensus sequence for HCV E2 by a proteolytic cleavage sequence. The consensus sequence for HCV E1 is SEQ ID NO:32. The consensus sequence for HCV E2 is SEQ ID NO:33.

In some embodiments, vaccines of the invention preferably include SEQ ID NO:32 and/or SEQ ID NO:33, or nucleic acid sequence which encode one of both of them. Vaccines of the invention preferably include SEQ ID NO:32 linked to SEQ ID NO:33 by a proteolytic cleavage sequence such as SEQ ID NO:29, or nucleic acid sequence which encodes the fusion protein. Vaccines of the present invention preferably include the IgE leader sequence SEQ ID NO:28.or nucleic acid sequence which encodes the same. Vaccines of the invention preferably include SEQ ID NO:31 or the nucleic acid sequence in SEQ ID NO:30.

In some embodiments of the invention, the vaccines of the invention include the SEQ ID NO:30 and a nucleic acid sequence or amino acid sequence encoded by the nucleic acid sequences thereof selected from the following group: SEQ ID NO:34, SEQ ID NO:35, and any combination thereof.

Fragments of SEQ ID NO:30 may comprise 30 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 45 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 60 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 75 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 120 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 150 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 180 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 210 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 240 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 270 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 300 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 360 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 420 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 480 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 540 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 600 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 660 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 720 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 780 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 840 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 900 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 960 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1020 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1080 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1140 or more nucleotides. In some

embodiments, fragments of SEQ ID NO:30 may comprise 1200 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1260 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1320 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1380 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1440 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1500 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1560 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1620 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1680 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise 1740 or more nucleotides. In some embodiments, fragments of SEQ ID NO:30 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:30 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 60 nucleotides, in some embodiments fewer than 75 nucleotides, in some embodiments fewer than 90 nucleotides, in some embodiments fewer than 120 nucleotides, in some embodiments fewer than 150 nucleotides, in some embodiments fewer than 180 nucleotides, in some embodiments fewer than 210 nucleotides, in some embodiments fewer than 240 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 300 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 420 nucleotides, in some embodiments fewer than 480 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 600 nucleotides, in some embodiments fewer than 660 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 780 nucleotides, in some embodiments fewer than 840 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 960 nucleotides, in some embodiments fewer than 1020 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1140 nucleotides, in some embodiments fewer than 1200 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1320 nucleotides, in some embodiments fewer than 1380 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1500 nucleotides, in some embodiments fewer than 1560 nucleotides, in some embodiments fewer than 1620 nucleotides, in some

embodiments fewer than 1680 nucleotides, and in some embodiments fewer than 1740 nucleotides.

Fragments of SEQ ID NO:31 may comprise 15 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 45 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 60 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 75 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 90 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 105 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 120 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 150 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 180 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 210 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 240 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 270 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 300 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 360 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 420 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 480 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 540 or more amino acids. In some embodiments, fragments of SEQ ID NO:31 may comprise 575 or more amino acids. Fragments may comprise fewer than 30 amino acids, in some embodiments fewer than 45 amino acids, in some embodiments fewer than 60 amino acids, in some embodiments fewer than 75 amino acids, in some embodiments fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 360 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 480 amino acids, in

some embodiments fewer than 540 amino acids, and in some embodiments fewer than 575 amino acids.

hTERT

hTERT is a human telomerase reverse transcriptase that synthesizes a TTAGGG tag on the end of telomeres to prevent cell death due to chromosomal shortening. Hyperproliferative cells with abnormally high expression of hTERT may be targeted by immunotherapy. Recent studies also support the abnormal expression of hTERT on hyperproliferative cells infected with HCV and HPV. Thus, immunotherapy for both HPV and HCV may be enhanced by targeting cells that express hTERT at abnormal levels.

Recent studies demonstrate that hTERT expression in dendritic cells transfected with hTERT genes can induce CD8+ cytotoxic T cells and elicit a CD4+ T cells in an antigen-specific fashion. Therefore, use of hTERT expression within antigen presenting cells (APCs) to delay senescence and sustain their capacity to present the antigen of choice is attractive in developing new methods of immunotherapy.

According to some embodiments of the invention, methods of inducing an immune response in individuals against an immunogen comprise administering to the individual the hTERT protein and functional fragments thereof or expressible coding sequences thereof in combination with an isolated nucleic acid molecule that encodes protein of the invention and/or a recombinant vaccine that encodes protein of the invention and/or a subunit vaccine that protein of the invention and/or a live attenuated vaccine and/or a killed vaccine.

In some embodiments of the invention, the vaccines of the invention include the SEQ ID NO:30 and a nucleic acid sequence or amino acid sequence encoded by the nucleic acid sequences thereof selected from the following group: SEQ ID NO:34, SEQ ID NO:35, and any combination thereof. In some embodiments of the invention, the vaccines of the invention comprise SEQ ID NO:34 or SEQ ID NO:35. SEQ ID NO:34 comprises the nucleic acid sequence that encodes hTERT. SEQ ID NO:35 comprises the amino acid sequence for hTERT.

In some embodiments of the invention, the vaccines of the invention comprise SEQ ID NO:22 and SEQ ID NO:34 or SEQ ID NO: 35. Using nucleic acid sequences that encode hTERT and/or protein of hTERT in combination with the HPV immunogens enhance the cell-mediated immune response against HPV-infected cells.

Fragments of SEQ ID NO:34 may comprise 30 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 45 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 60 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:1 may comprise 75 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 90 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 120 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 150 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 180 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 210 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 240 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 270 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 300 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 360 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 420 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 480 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 540 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 600 or more nucleotides, including preferably sequences that encode an immunodominant

epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 300 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 660 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 720 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 780 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 840 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 900 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 960 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 1020 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 1080 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1140 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 1200 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1260 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1320 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1380 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 1440 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 1500 or more nucleotides, including preferably sequences that encode an immunodominant epitope. . In some embodiments, fragments of SEQ ID NO:34 may comprise 1560 or more

nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1620 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1680 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1740 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1800 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1860 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1920 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 1980 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2040 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2100 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2160 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2220 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2280 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2340 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2400 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2460 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2520 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments

of SEQ ID NO:34 may comprise 2580 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2640 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2700 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2760 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2820 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2880 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 2940 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3000 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3060 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3120 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3180 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3240 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3300 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3360 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3420 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise 3480 or more nucleotides, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:34 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of

SEQ ID NO:34 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 60 nucleotides, in some embodiments fewer than 75 nucleotides, in some embodiments fewer than 90 nucleotides, in some embodiments fewer than 120 nucleotides, in some embodiments fewer than 150 nucleotides, in some embodiments fewer than 180 nucleotides, in some embodiments fewer than 210 nucleotides, in some embodiments fewer than 240 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 300 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 420 nucleotides, in some embodiments fewer than 480 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 600 nucleotides, in some embodiments fewer than 660 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 780 nucleotides, in some embodiments fewer than 840 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 960 nucleotides, in some embodiments fewer than 1020 nucleotides, in some embodiments fewer than 1080 nucleotides, in some embodiments fewer than 1140 nucleotides, in some embodiments fewer than 1200 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1320 nucleotides, in some embodiments fewer than 1380 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1500 nucleotides, in some embodiments fewer than 1560 nucleotides, in some embodiments fewer than 1620 nucleotides, in some embodiments fewer than 1680 nucleotides, in some embodiments fewer than 1740 nucleotides, in some embodiments fewer than 1800 nucleotides, in some embodiments fewer than 1860 nucleotides, in some embodiments fewer than 1920 nucleotides, in some embodiments fewer than 1980 nucleotides, in some embodiments fewer than 2040 nucleotides, in some embodiments fewer than 2100 nucleotides, in some embodiments fewer than 2160 nucleotides, in some embodiments fewer than 2220 nucleotides, in some embodiments fewer than 2280 nucleotides, in some embodiments fewer than 2340 nucleotides, in some embodiments fewer than 2400 nucleotides, in some embodiments fewer than 2460 nucleotides, in some embodiments fewer than 2520 nucleotides, in some embodiments fewer than 2580 nucleotides, in some embodiments fewer than 2640 nucleotides, in some embodiments fewer than 2700 nucleotides, in some embodiments fewer than 2760 nucleotides, in some embodiments fewer than 2820 nucleotides, in some embodiments fewer than 2860 nucleotides,

in some embodiments fewer than 2940 nucleotides, in some embodiments fewer than 3000 nucleotides, in some embodiments fewer than 3060 nucleotides, in some embodiments fewer than 3120 nucleotides, in some embodiments fewer than 3180 nucleotides, in some embodiments fewer than 3240 nucleotides, in some embodiments fewer than 3300 nucleotides, in some embodiments fewer than 3360 nucleotides, in some embodiments fewer than 3420 nucleotides, in some embodiments fewer than 3480 nucleotides, and in some embodiments fewer than 3510 nucleotides.

Fragments of SEQ ID NO:35 may comprise 15 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 18 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 21 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 24 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 30 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 36 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 42 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 48 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 54 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 60 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 66 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 72 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 90 or more amino acids, including preferably sequences that encode an immunodominant

epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 120 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 150 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 180 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 210 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 240 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 270 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 300 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 330 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 360 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 390 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 420 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 450 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 480 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 510 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 540 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 570 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 600 or more amino acids, including

preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 630 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 660 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 690 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 720 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 750 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 780 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 810 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 840 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 870 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 900 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 930 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 960 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 990 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1020 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1050 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1080 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may

comprise 1110 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1140 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1170 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1200 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1230 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1260 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1290 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1320 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1350 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1380 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1410 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1440 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1470 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise 1500 or more amino acids, including preferably sequences that encode an immunodominant epitope. In some embodiments, fragments of SEQ ID NO:35 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:35 do not comprise coding sequences for the IgE leader sequences. Fragments may comprise fewer than 24 amino acids, in some embodiments fewer than 30 amino acids, in some embodiments fewer than 36 amino acids, in some embodiments fewer than 42 amino acids, in some embodiments fewer than 48 amino acids, in

some embodiments fewer than 54 amino acids, in some embodiments fewer than 60 amino acids, in some embodiments fewer than 72 amino acids, in some embodiments fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids in some embodiments fewer than 240 amino acids, in some embodiments fewer than 260 amino acids, in some embodiments fewer than 290 amino acids, in some embodiments fewer than 320 amino acids, in some embodiments fewer than 350 amino acids, in some embodiments fewer than 380 amino acids, in some embodiments fewer than 410 amino acids in some embodiments fewer than 440 amino acids, in some embodiments fewer than 470 amino acids in some embodiments fewer than 500 amino acids, in some embodiments fewer than 530 amino acids in some embodiments fewer than 560 amino acids, in some embodiments fewer than 590 amino acids, in some embodiments fewer than 620 amino acids, in some embodiments fewer than 650 amino acids, in some embodiments fewer than 680 amino acids, in some embodiments fewer than 710 amino acids, in some embodiments fewer than 740 amino acids, in some embodiments fewer than 770 amino acids, in some embodiments fewer than 800 amino acids, in some embodiments fewer than 830 amino acids, in some embodiments fewer than 860 amino acids, in some embodiments fewer than 890 amino acids, in some embodiments fewer than 920 amino acids, in some embodiments fewer than 950 amino acids, in some embodiments fewer than 980 amino acids, in some embodiments fewer than 1010 amino acids, in some embodiments fewer than 1040 amino acids, in some embodiments fewer than 1070 amino acids, in some embodiments fewer than 1200 amino acids, in some embodiments fewer than 1230 amino acids, in some embodiments fewer than 1260 amino acids, in some embodiments fewer than 1290 amino acids, in some embodiments fewer than 1320 amino acids, in some embodiments fewer than 1350 amino acids, in some embodiments fewer than 1380 amino acids, in some embodiments fewer than 1410 amino acids, in some embodiments fewer than 1440 amino acids, in some embodiments fewer than 1470 amino acids, and in some embodiments fewer than 1500 amino acids.

Influenza

According to some embodiments of the invention, methods of inducing an immune response in individuals against an immunogen comprise administering to the individual the

Influenza strain H5N1 hemagglutinin (HA) protein and functional fragments thereof or expressible coding sequences thereof in combination with an isolated nucleic acid molecule that encodes protein of the invention and/or a recombinant vaccine that encodes protein of the invention and/or a subunit vaccine that protein of the invention and/or a live attenuated vaccine and/or a killed vaccine. In some embodiments, the Influenza vaccine compositions and methods comprise the use of a nucleic acid sequence that encodes HA protein from Influenza virus species. In some embodiments, the Influezna vaccine compositions and method comprise the use of nucleic acid sequences that encode HA from Influenza viral strain H1N5 and nucleic acid sequences encoding Influenza proteins selected from the group consisting of: SEQ ID NO:38, SEQ ID NO:40, and SEQ ID NO:42. In some embodiments of the invention, the vaccines of the invention comprise SEQ ID NO:36 or SEQ ID NO:37. SEQ ID NO:36 comprises the nucleic acid sequence that encodes H1N5 HA of Influenza virus. SEQ ID NO:37 comprises the amino acid sequence for H1N5 HA of Influenza virus. In some embodiments of the invention, the vaccines of the invention comprise SEQ ID NO:38 or SEQ ID NO:39. SEQ ID NO:38 comprises the nucleic acid sequence that encodes Influenza H1N1 and H5N1 NA consensus sequences. SEQ ID NO:39 comprises the amino acid sequence for Influenza H1N1 and H5N1 NA consensus sequences. In some embodiments of the invention, the vaccines of the invention comprise SEQ ID NO:40 or SEQ ID NO:41. SEQ ID NO:40 comprises the nucleic acid sequence that encodes Influenza H1N1 and H5N1 M1 consensus sequences. SEQ ID NO:41 comprises the amino acid sequence for Influenza H1N1 and H5N1 M1 consensus sequences. In some embodiments of the invention, the vaccines of the invention comprise SEQ ID NO:42 or SEQ ID NO:43. SEQ ID NO:42 comprises the nucleic acid sequence that encodes Influenza H5N1 M2E-NP consensus sequence. SEQ ID NO:43 comprises the amino acid sequence for Influenza H5N1 M2E-NP consensus sequence. In some embodiments of the invention, the vaccines of the invention include the SEQ ID NO:36 and a sequence selected from the following group: SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, and any combination thereof. The consensus sequence for Influenza virus strain H5N1 HA includes the immunodominant epitope set forth in SEQ ID NO:36. The Influenza virus H5N1 HA amino acid sequence encoded by SEQ ID NO:36 is SEQ ID NO:37. The consensus sequence for Influenza virus H1N1/H5N1 NA includes the immunodominant

epitope set forth in SEQ ID NO:38. The Influenza virus strains H1N1/H5N1 NA amino acid sequence encoded by SEQ ID NO:38 is SEQ ID NO:39. The consensus sequence for Influenza virus strains H1N1/H5N1 M1 includes the immunodominant epitope set forth in SEQ ID NO:40. The Influenza virus H1N1/H5N1 M1 amino acid sequence encoded by SEQ ID NO:40 is SEQ ID NO:41. The consensus sequence for Influenza virus H5N1 M2E-NP includes the immunodominant epitope set forth in SEQ ID NO:42. The Influenza virus H5N1 M2E-NP amino acid sequence encoded by SEQ ID NO:42 is SEQ ID NO:43. Vaccines of the present invention may include protein products encoded by the nucleic acid molecules defined above or any fragments of proteins.

Fragments of SEQ ID NO:36 may comprise 30 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 45 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 60 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 75 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 90 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 120 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 150 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 180 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 210 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 240 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 270 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 300 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 360 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 420 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 480 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 540 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 600 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 660 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 720 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 780 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 840 or more nucleotides. In some

embodiments, fragments of SEQ ID NO:36 may comprise 900 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 960 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1020 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1080 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1140 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1200 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1260 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1320 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1380 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1440 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1500 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1560 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1620 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1680 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise 1740 or more nucleotides. In some embodiments, fragments of SEQ ID NO:36 may comprise coding sequences for the IgE leader sequences. In some embodiments, fragments of SEQ ID NO:36 do not comprise coding sequences for the IgE leader sequences. Fragments of SEQ ID NO:36 may comprise fewer than 60 nucleotides, in some embodiments fewer than 75 nucleotides, in some embodiments fewer than 90 nucleotides, in some embodiments fewer than 120 nucleotides, in some embodiments fewer than 150 nucleotides, in some embodiments fewer than 180 nucleotides, in some embodiments fewer than 210 nucleotides, in some embodiments fewer than 240 nucleotides, in some embodiments fewer than 270 nucleotides, in some embodiments fewer than 300 nucleotides, in some embodiments fewer than 360 nucleotides, in some embodiments fewer than 420 nucleotides, in some embodiments fewer than 480 nucleotides, in some embodiments fewer than 540 nucleotides, in some embodiments fewer than 600 nucleotides, in some embodiments fewer than 660 nucleotides, in some embodiments fewer than 720 nucleotides, in some embodiments fewer than 780 nucleotides, in some embodiments fewer than 840 nucleotides, in some embodiments fewer than 900 nucleotides, in some embodiments fewer than 960 nucleotides, in some embodiments fewer than 1020 nucleotides, in some embodiments fewer

than 1080 nucleotides, in some embodiments fewer than 1140 nucleotides, in some embodiments fewer than 1200 nucleotides, in some embodiments fewer than 1260 nucleotides, in some embodiments fewer than 1320 nucleotides, in some embodiments fewer than 1380 nucleotides, in some embodiments fewer than 1440 nucleotides, in some embodiments fewer than 1500 nucleotides, in some embodiments fewer than 1560 nucleotides, in some embodiments fewer than 1620 nucleotides, in some embodiments fewer than 1680 nucleotides, and in some embodiments fewer than 1740 nucleotides.

Fragments of SEQ ID NO:37 may comprise 15 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 30 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 45 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 60 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 75 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 90 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 105 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 120 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 150 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 180 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 210 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 240 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 270 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 300 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 360 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 420 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 480 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 540 or more amino acids. In some embodiments, fragments of SEQ ID NO:37 may comprise 565 or more amino acids. Fragments of SEQ ID NO:37 may comprise fewer than 30 amino acids, in some embodiments fewer than 45 amino acids, in some embodiments fewer than 60 amino acids, in some embodiments fewer than 75 amino acids, in some embodiments fewer than 90 amino acids, in some embodiments fewer than 120 amino acids, in some embodiments fewer than 150 amino acids, in some

embodiments fewer than 180 amino acids, in some embodiments fewer than 210 amino acids, in some embodiments fewer than 240 amino acids, in some embodiments fewer than 270 amino acids, in some embodiments fewer than 300 amino acids, in some embodiments fewer than 360 amino acids, in some embodiments fewer than 420 amino acids, in some embodiments fewer than 480 amino acids, in some embodiments fewer than 540 amino acids, and in some embodiments fewer than 565 amino acids.

According to some embodiments of the invention, methods of inducing an immune response in individuals against an immunogen comprise administering to the individual the Influenza strain H1N1 and Influenza strain H5N1 NA protein and functional fragments thereof or expressible coding sequences thereof in combination with an isolated nucleic acid molecule that encodes protein of the invention and/or a recombinant vaccine that encodes protein of the invention and/or a subunit vaccine that protein of the invention and/or a live attenuated vaccine and/or a killed vaccine.

According to some embodiments of the invention, methods of inducing an immune response in individuals against an immunogen comprise administering to the individual the Influenza strain H1N1 and Influenza strain H5N1 M1 protein and functional fragments thereof or expressible coding sequences thereof in combination with an isolated nucleic acid molecule that encodes protein of the invention and/or a recombinant vaccine that encodes protein of the invention and/or a subunit vaccine that protein of the invention and/or a live attenuated vaccine and/or a killed vaccine.

According to some embodiments of the invention, methods of inducing an immune response in individuals against an immunogen comprise administering to the individual the Influenza strain H5N1 M2E-NP protein and functional fragments thereof or expressible coding sequences thereof in combination with an isolated nucleic acid molecule that encodes protein of the invention and/or a recombinant vaccine that encodes protein of the invention and/or a subunit vaccine that protein of the invention and/or a live attenuated vaccine and/or a killed vaccine.

Vaccines

The invention provides improved vaccines by providing proteins and genetic constructs that encode proteins with epitopes that make them particularly effective as immunogens against which immune responses can be induced. Accordingly, vaccines can be provided to induce a

therapeutic or prophylactic immune response. In some embodiments, the means to deliver the immunogen is a DNA vaccine, a recombinant vaccine, a protein subunit vaccine, a composition comprising the immunogen, an attenuated vaccine or a killed vaccine. In some embodiments, the vaccine comprises a combination selected from the groups consisting of: one or more DNA vaccines, one or more recombinant vaccines, one or more protein subunit vaccines, one or more compositions comprising the immunogen, one or more attenuated vaccines and one or more killed vaccines.

According to some embodiments of the invention, a vaccine according to the invention is delivered to an individual to modulate the activity of the individual's immune system and thereby enhance the immune response. When a nucleic acid molecules that encodes the protein is taken up by cells of the individual the nucleotide sequence is expressed in the cells and the protein are thereby delivered to the individual. Aspects of the invention provide methods of delivering the coding sequences of the protein on nucleic acid molecule such as plasmid, as part of recombinant vaccines and as part of attenuated vaccines, as isolated proteins or proteins part of a vector.

According to some aspects of the present invention, compositions and methods are provided which prophylactically and/or therapeutically immunize an individual

DNA vaccines are described in US. Patent Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, 5,676,594, and the priority applications cited therein, which are each incorporated herein by reference. In addition to the delivery protocols described in those applications, alternative methods of delivering DNA are described in US. Patent Nos. 4,945,050 and 5,036,006, which are both incorporated herein by reference.

The present invention relates to improved attenuated live vaccines, improved killed vaccines and improved vaccines that use recombinant vectors to deliver foreign genes that encode antigens and well as subunit and glycoprotein vaccines. Examples of attenuated live vaccines, those using recombinant vectors to deliver foreign antigens, subunit vaccines and glycoprotein vaccines are described in U.S. Patent Nos.: 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,3 64; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439;

5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

When taken up by a cell, the genetic construct(s) may remain present in the cell as a. functioning extrachromosomal molecule and/or integrate into the cell's chromosomal DNA. DNA may be introduced into cells where it remains as separate genetic material in the form of a plasmid or plasmids. Alternatively, linear DNA that can integrate into the chromosome may be introduced into the cell. When introducing DNA into the cell, reagents that promote DNA integration into chromosomes may be added. DNA sequences that are useful to promote integration may also be included in the DNA molecule. Alternatively, RNA may be administered to the cell. It is also contemplated to provide the genetic construct as a linear minichromosome including a centromere, telomeres and an origin of replication. Gene constructs may remain part of the genetic material in attenuated live microorganisms or recombinant microbial vectors which live in cells. Gene constructs may be part of genomes of recombinant viral vaccines where the genetic material either integrates into the chromosome of the cell or remains extrachromosomal. Genetic constructs include regulatory elements necessary for gene expression of a nucleic acid molecule. The elements include: a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers are often required for gene expression of the sequence that encodes the target protein or the immunomodulating protein. It is necessary that these elements be operable linked to the sequence that encodes the desired proteins and that the regulatory elements are operably in the individual to whom they are administered.

Initiation codons and stop codon are generally considered to be part of a nucleotide sequence that encodes the desired protein. However, it is necessary that these elements are functional in the individual to whom the gene construct is administered. The initiation and termination codons must be in frame with the coding sequence.

Promoters and polyadenylation signals used must be functional within the cells of the individual.

Examples of promoters useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to promoters from Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (MV) such as the BIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV,

Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human Actin, human Myosin, human Hemoglobin, human muscle creatine and human metalothionein.

Examples of polyadenylation signals useful to practice the present invention, especially in the production of a genetic vaccine for humans, include but are not limited to SV40 polyadenylation signals and LTR polyadenylation signals. In particular, the SV40 polyadenylation signal that is in pCEP4 plasmid (Invitrogen, San Diego CA), referred to as the SV40 polyadenylation signal, is used.

In addition to the regulatory elements required for DNA expression, other elements may also be included in the DNA molecule. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human Actin, human Myosin, human Hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

Genetic constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. Plasmids pVAX1, pCEP4 and pREP4 from Invitrogen (San Diego, CA) contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region which produces high copy episomal replication without integration.

In some preferred embodiments related to immunization applications, nucleic acid molecule(s) are delivered which include nucleotide sequences that encode protein of the invention , and, additionally, genes for proteins which further enhance the immune response against such target proteins. Examples of such genes are those which encode other cytokines and lymphokines such as alpha-interferon, gamma-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, MHC, CD80,CD86 and IL- 15 including IL-15 having the signal sequence deleted and optionally including the signal peptide from IgE. Other genes which may be useful include those encoding: MCP-1, MIP-lα, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt,

Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof

An additional element may be added which serves as a target for cell destruction if it is desirable to eliminate cells receiving the genetic construct for any reason. A herpes thymidine kinase (tk) gene in an expressible form can be included in the genetic construct. The drug gangcyclovir can be administered to the individual and that drug will cause the selective killing of any cell producing tk, thus, providing the means for the selective destruction of cells with the genetic construct.

In order to maximize protein production, regulatory sequences may be selected which are well suited for gene expression in the cells the construct is administered into. Moreover, codons may be selected which are most efficiently transcribed in the cell. One having ordinary skill in the art can produce DNA constructs that are functional in the cells.

In some embodiments, gene constructs may be provided in which the coding sequences for the proteins described herein are linked to IgE signal peptide. In some embodiments, proteins described herein are linked to IgE signal peptide.

In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, produce and isolate proteins of the invention using well known techniques. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, inserts DNA molecules that encode a protein of the invention into a commercially available expression vector for use in well known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, Calif.) may be used for production of protein in E. coli. The commercially available plasmid pYES2 (Invitrogen, San Diego, Calif.) may, for example, be used for production in S. cerevisiae strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA I or pcDNA3

(Invitrogen, San Diego, Calif.) may, for example, be used for production in mammalian cells such as Chinese Hamster Ovary cells. One having ordinary skill in the art can use these commercial expression vectors and systems or others to produce protein by routine techniques and readily available starting materials. (See e.g., Sambrook et al., Molecular Cloning a Laboratory Manual, Second Ed. Cold Spring Harbor Press (1989) which is incorporated herein by reference.) Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

One having ordinary skill in the art may use other commercially available expression vectors and systems or produce vectors using well known methods and readily available starting materials. Expression systems containing the requisite control sequences, such as promoters and polyadenylation signals, and preferably enhancers are readily available and known in the art for a variety of hosts. See e.g., Sambrook et al., Molecular Cloning a Laboratory Manual, Second Ed. Cold Spring Harbor Press (1989). Genetic constructs include the protein coding sequence operably linked to a promoter that is functional in the cell line into which the constructs are transfected. Examples of constitutive promoters include promoters from cytomegalovirus or SV40. Examples of inducible promoters include mouse mammary leukemia virus or metallothionein promoters. Those having ordinary skill in the art can readily produce genetic constructs useful for transfecting with cells with DNA that encodes protein of the invention from readily available starting materials. The expression vector including the DNA that encodes the protein is used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign DNA takes place.

The protein produced is recovered from the culture, either by lysing the cells or from the culture medium as appropriate and known to those in the art. One having ordinary skill in the art can, using well known techniques, isolate protein that is produced using such expression systems. The methods of purifying protein from natural sources using antibodies which specifically bind to a specific protein as described above may be equally applied to purifying protein produced by recombinant DNA methodology.

In addition to producing proteins by recombinant techniques, automated peptide synthesizers may also be employed to produce isolated, essentially pure protein. Such techniques

are well known to those having ordinary skill in the art and are useful if derivatives which have substitutions not provided for in DNA-encoded protein production.

The nucleic acid molecules may be delivered using any of several well known technologies including DNA injection (also referred to as DNA vaccination), recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia.

Routes of administration include, but are not limited to, intramuscular, intransally, intraperitoneal, intradermal, subcutaneous, intravenous, intraarterially, intraoccularly and oral as well as topically, transdermally, by inhalation or suppository or to mucosal tissue such as by lavage to vaginal, rectal, urethral, buccal and sublingual tissue. Preferred routes of administration include intramuscular, intraperitoneal, intradermal and subcutaneous injection. Genetic constructs may be administered by means including, but not limited to, traditional syringes, needleless injection devices, or "microprojectile bombardment gone guns".

In some embodiments, the nucleic acid molecule is delivered to the cells in conjunction with administration of a polynucleotide function enhancer or a genetic vaccine facilitator agent. Polynucleotide function enhancers are described in U.S. Serial Number 5,593,972, 5,962,428 and International Application Serial Number PCT/US94/00899 filed January 26, 1994, which are each incorporated herein by reference. Genetic vaccine facilitator agents are described in US. Serial Number 021,579 filed April 1, 1994, which is incorporated herein by reference. The co-agents that are administered in conjunction with nucleic acid molecules may be administered as a mixture with the nucleic acid molecule or administered separately simultaneously, before or after administration of nucleic acid molecules. In addition, other agents which may function transfecting agents and/or replicating agents and/or inflammatory agents and which may be co-administered with a GVF include growth factors, cytokines and lymphokines such as *a*-interferon, gamma-interferon, GM-CSF, platelet derived growth factor (PDGF), TNF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-10, IL-12 and IL-15 as well as fibroblast growth factor, surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl Lipid A (WL), muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the genetic construct In some embodiments, an

immunomodulating protein may be used as a GVF. In some embodiments, the nucleic acid molecule is provided in association with PLG to enhance delivery/uptake.

The pharmaceutical compositions according to the present invention comprise about 1 nanogram to about 2000 micrograms of DNA. In some preferred embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 10 nanograms to about 800 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 100 to about 200 microgram DNA.

The pharmaceutical compositions according to the present invention are formulated according to the mode of administration to be used. In cases where pharmaceutical compositions are injectable pharmaceutical compositions, they are sterile, pyrogen free and particulate free. An isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. In some embodiments, a vasoconstriction agent is added to the formulation.

According to some embodiments of the invention, methods of inducing immune responses are provided. The vaccine may be a protein based, live attenuated vaccine, a cell vaccine, a recombinant vaccine or a nucleic acid or DNA vaccine. In some embodiments, methods of inducing an immune response in individuals against an immunogen, including methods of inducing mucosal immune responses, comprise administering to the individual one or more of CTACK protein, TECK protein, MEC protein and functional fragments thereof or expressible coding sequences thereof in combination with an isolated nucleic acid molecule that encodes protein of the invention and/or a recombinant vaccine that encodes protein of the invention and/or a subunit vaccine that protein of the invention and/or a live attenuated vaccine and/or a killed vaccine. The one or more of CTACK protein, TECK protein, MEC protein and functional fragments thereof may be administered prior to, simultaneously with or after

administration of the isolated nucleic acid molecule that encodes an immunogen; and/or recombinant vaccine that encodes an immunogen and/or subunit vaccine that comprises an immunogen and/or live attenuated vaccine and/or killed vaccine. In some embodiments, an isolated nucleic acid molecule that encodes one or more proteins of selected from the group consisting of: CTACK, TECK, MEC and functional fragments thereof is administered to the individual.

EXAMPLES

Example 1

MATERIALS AND METHODS

HIV-1 subtype B envelope sequences. To generate HIV-1 subtype B consensus envelope sequence, forty-two subtype B envelope gene sequences collected from eleven countries were selected from GenBank to avoid sampling bias. Each sequence represents a different patient. All sequences used are non-recombinant.

Multiple alignment. The alignment procedure applied in the phylogenetic study included the application of Clustal X (version 1.81) (Thompson, J. D., et al. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876-4882). Pairwise alignment parameters were set to the dynamic "slow-accurate" programming, using 10 as the gap opening penalty and 0.1 as the gap extension penalty. Multiple alignment parameters included a gap extension penalty equal to 0.2.

Construction of HIV-1 subtype B envelope consensus sequence. The HIV-1 subtype B envelope consensus nucleotide sequence was obtained after performing multiple alignment and minor final manual adjustment. Deduced amino acid sequences were used to guide the introduction of alignment gaps so that they were inserted between codons. The consensus amino acid sequence was obtained by translating the consensus nucleotide sequence.

Phylogenetic tree. The neighbor-joining (NJ) method was employed for amino acid phylogenetic tree-building using the program PAUP* 4.0b10 (Swofford, D. L. 1999. PAUP* 4.0: phylogenetic analysis using parsimony (* and other methods), version 4.0b2a. Sinauer Associates, Inc.,, Sunderland, Mass.). Two additional sequences from subtype D (K03454 and AAA44873) and two sequences from subtype C (AAD12103 and AAD12112) were used as an

outgroup for rooting (Kuiken, C., B. T. Korber, and R. W. Shafer. 2003. HIV sequence databases. AIDS Rev. 5:52-61).

Modifications of HIV-1 subtype B envelope consensus sequence. Several modifications were performed after obtaining HIV-1 subtype B consensus envelope sequence: highly variable V1 and V2 regions were shortened, V3 loop was designed for CCR5 utilization, the cytoplasmic tail region was removed from the C-terminal, a leader sequence and an upstream Kozak sequence were added to the N-terminal, codon optimization and RNA optimization was performed by using GeneOptimizerTM (GENEART, Germany).

Envelope Immunogens. The gene encoding modified HIV-1 subtype B early transmitter consensus envelope glycoprotein (EY2E1-B) was synthesized and sequence verified by GENEART. The synthesized EY2E1-B was digested with BamHI and NotI, cloned into the expression vector pVAX (Invitrogen) under the control of the cytomegalovirus immediate-early promoter and this construct was named as pEY2E1-B.

The primary subtype B immunogen (EK2P-B) was generated from a human codon biased, primary subtype B isolate 6101 gp140 envelope gene that was a gift of M. Sidhm (Wyeth). Basically, the optimized 6101 envelope gene was mutated by removing the native leader sequence and cytoplasmic tail. Then the IgE-leader sequence and Kozak sequence were introduced by designing forward and reverse specific- primers: Env-F: 5'-GTCGCTCCGCTAGCTTGTGGGTCACAGTCTATTATGGGGTACC-3' (SEQ ID NO:13) Env-R: 5'-GGTCGGATCCTTACTCCACCACTCTCCTTTTTGCC-3' (SEQ ID NO:14). The purified PCR product was cloned into pVAX plasmid vector, which was also linearized with EcoR1 and XbaI. This construct was named as pEK2P-B.

In vivo Expression and Reactivity of EY2E1-B with Monoclonal Antibodies. Human rhabdomyosarcoma (RD) cells (2 x 106) were transfected in 60 mm dishes with 3 □g of pEY2E1-B and pEK2P-B plasmids using FuGENE 6 Transfection Reagent (Roche, Germany), respectively. Forty-eight hours after transfection, cells were washed three times with 1 x PBS and lysed in 150 µl of lysis buffer (Cell Signaling Technology). The total protein lysates (50 µg) were fractioned on a SDS-PAGE gel, transferred to a PVDF membrane (Amersham). Immunoblot analyses were performed with an envelope-specific monoclonal antibody 2G12 (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) and a monoclonal

anti-actin antibody (Sigma-Aldrich) and visualized with HRP-conjugated goat anti-human IgG (Sigma- Aldrich) using an ECLTM Western blot analysis system (Amersham). Actin was used as a loading control for Western Blot.

To detect the reactivity of EY2E1-B with monoclonal antibodies, the total protein lysates from transfection (100 μg) were immunoprecipitated with 5 μg envelope-specific monoclonal antibodies including 2G12, 4G10 and ID6 (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA). The same amount of total protein lysates from cells transfected with empty vector pVAX was used as a negative control. The immunoprecipitated proteins were fractioned on a SDS-PAGE gel and detected by Western Blotting described as above.

Indirect Immunofluorescent Assay. An indirect immunofluorescent assay for confirming the expression of EY2E1-B and EK2P-B genes was performed. Human rhabdomyosarcoma (RD) cells were plated in tissue culture chambered slides (BD Biosciences), at a density to obtain 60-70% confluency the next day in complete DMEM medium with 10% FBS (GIBCO) and allow to adhere overnight. The next day cells were transfected with pEY2E1-B, pEK2P-B and the control plasmid pVAX (1 μg/well) using FuGENE 6 Transfection Reagent (Roche) according to the manufacturer's instructions. Forty-eight hours after transfection, the cells were washed twice with cold 1XPBS and fixed on slides using methanol for 15 min. Upon removal of the residual solvents from the slides, the cells were incubated with anti-mouse HIV-1 env monoclonal F105 (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA) for 90 min. The slides were then incubated with TRITC-conjugated secondary antibody (Sigma-Aldrich) for 45 min. 4', 6-Diamido-2-phenylindole hydrochloride (Sigma-Aldrich) was added to the solution of secondary antibody to counter stain nuclei to show the nuclei of the total number of cells available in the given field. The slides were mounted with mounting medium containing antifading reagent (Molecular Probes). The images were analyzed using the Phase 3 Pro program for fluorescent microscopy (Media Cybernetics).

Envelope-specific Antibody determination The measurement of IgG antibodies specific for Envelope was performed by ELISA (enzyme linked immunosorbent assay) in both immunized and control mice. Nunc-ImmunoTM Plates (Nalge Nunc International, Rochester, NY) were coated with 1μg/ml of clade B recombinant HIV-1 IIIB glycoprotein soluble gp160 (Immuno Diagnostics, MA), clade A/E primary envelope protein HIV-1 93TH975 gp120 and

clade C primary envelope protein HIV-1 96ZM651 gp120 (NIH AIDS Research and Reference Reagent Program, Rockville, MD, USA), respectively, and incubated overnight at room temperature. After washing, plates were blocked with 3% BSA in PBST (1 x PBS + 0.05% Tween-20) for 1 h at 37°C. Then plates were washed again and incubated with the specific mouse sera, diluted with 3% BSA in PBST overnight at 4°C, followed by incubation with a 1/10,000 dilution of HRP-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) for 1 h at 37°C. The reaction was developed with the substrate TMB (3, 3□, 5, 5□ -tetramethylbenzidine) (Sigma-Aldrich). Reaction was stopped with 100 µl of 2.5M sulfuric acid per well and the plates were read on the EL808 plate reader (Biotech Instrument Inc.) at OD of 450 nm.

Immunization of Mice Female 4–6-week-old BALB/c mice were purchased from The Jackson Laboratory, Bar Harbor, ME. The breeding pairs of transgenic B6.Cg-Tg (HLA-A/H2-D)2Enge/J mice were purchased from the Jackson Laboratory and bred by Dr. Michelle Kutzler in our lab. These transgenic mice express an interspecies hybrid class I MHC gene, AAD, which contains the alpha-1 and alpha-2 domains of the human HLA-A2.1 gene and the alpha-3 transmembrane and cytoplasmic domains of the mouse H-2Dd gene, under the direction of the human HLA-A2.1 promoter. The mouse alpha–3 domain expression enhances the immune response in this system. Compared to unmodified HLA-A2.1, the chimeric HLA-A2.1/H2-Dd MHC Class I molecule mediated efficient positive selection of mouse T cells to provide a more complete T cell repertoire capable of recognizing peptides presented by HLA-A2.1 Class I molecules. The peptide epitopes presented and recognized by mouse T cells in the context of the HLA-A2.1 Class I molecule are the same as those presented in HLA-A2.1+ humans. The female 4-6-week-old transgenic mice were used for further study described below. Their care was in accordance with the guidelines of the National Institutes of Health and the University of Pennsylvania Institutional Care and Use Committee (IACUC). Each mouse was immunized intramuscularly with three times, each of 100 µg of DNA at biweekly intervals. There are three mice in each group and the control group was vaccinated with pVAX DNA. Mice were sacrificed one week after the third immunization and the spleens were removed aseptically. The spleen cells were collected and resuspended in RBC lysis buffer to remove erythrocytes. After

lysis, the spleenocytes from the same group were pooled and resuspended in RPMI 1640 medium with 10% FBS. Cells were counted and prepared for analysis.

IFN-γ ELISpot Assay. High-Protein Binding IP 96 well Multiscreen™ plates (Millipore, Bedford, MA, USA) were used. Plates were coated with mAb to mouse IFN-γ (R&D Systems, Minneapolis, MN) diluted in 1XPBS, overnight at 4°C. Plates were washed three times with PBS and then blocked for 2 h at room temperature with 1XPBS supplemented with 1% BSA and 5% sucrose. Mice Splenocytes were added in triplicates at an input cell number of 2 x 10^5 cells per well resuspended in complete culture medium (RPMI 1640 supplemented with 10% FBS and antibiotics). Six sets of peptides each containing 15 amino acid residues overlapping by 11 amino acids representing the entire protein consensus sequences of HIV-1 subtype B, subtype C, group M and the entire protein sequences of HIV-1 MN (a subtype B isolate), HIV-1 C.UY.01.TRA3011 and C.ZA.01.J54Ma (two subtype C isolates) envelope were obtained from NIH AIDS Research and Reference Reagent Program. Each set of env peptides were pooled at a concentration of 2 μg/ml/peptide into 4 pools as antigens for specific stimulation of the IFN-γ release. Concavalin A (Sigma–Aldrich, St. Louis, MO), at 5 g/ml, and complete culture medium were used as positive and negative control, respectively. Plates were washed four times after a 24 h incubation at 37°C, in a 5% CO_2 atmosphere incubator. Then, a biotinilated anti-mouse IFN-γ detection antibody was added, and plates were incubated overnight at 4°C. The plates were washed, and color development was followed according to the manufacturer's instructions (ELISPOT Blue Color Module, R&D Systems, Minneapolis, MN). Plates were air-dried and the spots were counted using an automated ELISPOT reader system (CTL Analyzers, Cleveland, OH) with the ImmnunoSpot® software. The average number of spot forming cells (SFC) was adjusted to 1 x 10^6 splenocytes for data display. The ELISpot assay was repeated three times in three separate experiments.

CD8+ T-cell depletion study. CD8 lymphocytes were depleted from splenocytes by using immune-magnetic beads coated with antibody to CD8 (Dynal Biotech Inc., Lake Success, NY) following manufacturer's instructions. After depletion of CD8+ T-cells, IFN-γ ELISpot assay was performed as described above.

Epitope mapping study. In order to map the reactive epitopes, two sets of peptides containing 15 amino acid residues overlapping by 11 amino acids representing the entire

envelope proteins of HIV-1 consensus subtype B and HIV-1 MN were pooled into 29 pools of 14-15 peptides/per pool, respectively, and IFN-γ ELISpot assay was performed as described above. These different sets of 29 pooled stimulators were used in a matrix assay which facilitates epitope mapping.

Statistical Analysis. Student paired t-test was used for comparison of the cellular immune response between mice immunized with pEY2E1-B and pEK2P-B. In this study, $p<0.05$ has been considered statistically significant.

RESULTS

Construction and design of a novel subtype B early transmitter consensus-based envelope gene. The consensus sequence of HIV-1 subtype B was generated from 42 subtype B sequences retrieved from GenBank. As summarized in Fig. 1, several modifications were carried out after generating the consensus sequence. Briefly, to produce a CCR5-tropic version of HIV-1 envelope that mimicked mucosally transmitted viruses, six important amino acids in the V3 loop were designed according to the sequences of early transmitter isolates. Further, ten amino acids in V1 loop and one amino acid in V2 loop was also deleted from the consensus sequence. A highly efficient leader sequence was fused in frame upstream of the start codon to facilitate the expression. The transmembrane domain was kept intact to facilitate surface expression and the cleavage site was kept intact to obtain proper folding and host proteinase cleavage of the envelope protein. The cytoplasmic tail was removed to prevent envelope recycling and to promote more stable and higher surface expression (Berlioz-Torrent, C., et al. 1999. Interactions of the cytoplasmic domains of human and simian retroviral transmembrane proteins with components of the clathrin adaptor complexes modulate intracellular and cell surface expression of envelope glycoproteins. J. Virol. 73:1350-1359; Bultmann, A., et al.. 2001. Identification of two sequences in the cytoplamic tail of the human immunodeficiency virus type 1 envelope glycoprotein that inhibit cell surface expression. J. Virol. 75:5263-5276). Furthermore, in order to have a higher level of expression, the codon usage of this gene was adapted to the codon bias of Homo Sapiens genes (Andre, S., et al. B. 1998. Increased immune response elicited by DNA vaccination with a synthetic gp120 sequence with optimized codon usage. J Virol 72:1497-503; Deml, L., et al. 2001. Multiple effects of codon usage optimization on expression and immunogenicity of DNA candidate vaccines encoding the human immunodeficiency virus type 1

gag protein. J. Virol. 75:10991-11001). In addition, RNA optimization (Schneider, R., et al.. 1997. Inactivation of the human immunodeficiency virus type 1 inhibitory elements allows Rev-independent expression of Gag and Gag/protease and particle formation. J. Virol. 71:4892-4903) was also performed: regions of very high (>80%) or very low (<30%) GC content and the cis-acting sequence motifs such as internal TATA boxes, chi-sites and ribosomal entry sites were avoided. The synthetic engineered EY2E1-B gene was constructed and was 2734 bp in length. The EY2E1-B gene was subcloned into pVAX at the BamHI and NotI sites for further study.

Phylogenetic analysis. To assess the distribution of the distance from a randomly sampled envelope subtype B sequence to the EY2E1-B sequence, a phylogenetic analysis was performed. As shown in Fig. 2, there was an observed relative closeness of the EY2E1-B sequence to all sampled sequences. The EY2E1-B sequence, when compared with the primary isolate EK2P-B sequence, has comparable distributions of similarity scores (Table 1). The average percent similarity score for EY2E1-B was 85.7%, while it was 79.4% for EK2P-B.

Table 1

	Average percent similarity scores	Range of percent similarity scores
EY2E1-B	85.7	92.1-79.6
EK2P-B	79.4	86.3-73.9

Table 1. The average and range of percent similarity scores between potential envelope vaccine candidates and an alignment of subtype B envelope sequences.

In Vivo Expression and Antigenic Determination of EY2E1-B. In order to test the in vivo expression of pEY2E1-B and pEK2P-B, RD cells were transfected with these plasmids as described in Materials and Methods section. Total proteins were extracted from cell lysates after transfection and immunoblotted with the envelope-specific monoclonal antibody 2G12 mentioned in Materials and Methods section to detect the expression of pEY2E1-B. Western blot results indicated that these two constructs expressed envelope protein (Fig. 3A). The envelope protein detected was about 120 KD. Table 2 shows a comparison of pEY2E1-B and pEK2P-B.

Table 2

	Consensus/ Primary	Early transmitter	Codon-optimized	RNA-optimized	IgELS	Cytoplasmic tail
EY2E1-B	Consensus	Yes	Yes	Yes	Yes	No
EK2P-B	Primary	No	Yes	Yes	Yes	No

To determine the antigenic epitopes, the expressed envelope proteins from the RD cell lysates were immunoprecipitated with three different gp120-specific antibodies 2G12, 4G10 and ID6. Following the immunoprecipitation, Western Blotting was performed to detect the immnoprecipitated proteins. Our results showed that the synthetic immunogen could bind to antibodies 2G12 and ID6, but not 4G10. Since antibody 2G12 neutralizes a broad variety of primary isolates and reacts with a conformational and carbohydrate-dependent gp120 epitope, and antibody ID6 binds to gp120 and gp160 and is directed against the first 204 aa of gp120, our results suggested that the synthetic engineered immunogen EY2E1-B might be able to fold into a relatively native conformation and preserve some native antigenic epitopes. Furthermore, since the antibody 4G10 is a HIV-1 LAI/BRU V3 monoclonal antibody that recognizes LAI gp160, a T-cell line adapted strain, our data also suggested that this synthetic envelope would not utilize the coreceptor CXCR4.

To further confirm the expression and determine the antigenic epitopes, an indirect immunofluorescent assay was performed using transfected RD cells. High specific expression was observed under fluorescent microscope in the pEY2E1-B and pEK2P-B transfected cells. The HIV-1 env monoclonal F105 that reacts with a discontinuous, or conformational, gp120 epitope was used in the assay. As indicated in Fig. 3B, the transfected cells expressing Env proteins showed the typical rhodamine fluorescence, again suggesting the synthetic protein expressed and had a relatively native conformation. As a control, the expression was not detected in pVAX transfected RD cells.

Induction of humoral response. To determine whether the synthetic immunogen could elicit higher-titer envelope-specific antibody response, sera were collected from BalB/C mice immunized pVAX, pEY2E1-B and pEK2P-B and ELISA was performed. As shown in Fig. 4A, we observed the relatively higher level of clade B envelope-specific antibody responses with sera collected from pEY2E1-B immunized mice compared to these in pEK2P-B immunized mice. In contract, the vector alone mice didn't develop specific antibody responses. However, there were

not any detectable antibody responses against clade A/E and clade C proteins in both pEY2E1-B and pEK2P-B injected mice (Fig. 4B and 4C), indicating that although the synthetic consensus-based immunogen has a relatively native conformation and preserve native antigenic epitopes, it may not be able to induce broad cross-clade antibody immune responses.

Strong and broad cellular immune responses measured by ELISpot. The BalB/C mice were immunized with pEY2E1-B and pEK2P-B and ELISpot analysis was performed to determine the number of antigen-specific IFN-γ secreting cells in response to four pools of peptides from HIV-1 consensus subtype B protein (Fig. 5A). The magnitude of the response as measured by the number of spot forming units (SFU) per million cells ranged from 27.5 to 520 in pEY2E1-B vaccinated mice. In comparison, splenocytes from pEK2P-B vaccinated mice only showed the range of spots from 2 to 237.5 ($p<0.05$). The additive frequency of SFU/per million splenocytes for all four pools in pEY2E1-B immunized mice was 1976.25 + 260, while the number of SFU/per million cells in pEK2P-B immunized mice was 519 + 45. Cells from mice immunized with pVAX vector were used as a negative control, showing only 60 + 5 SFU/per million splenocytes for consensus envelope B peptides pools ($p < 0.05$). We observed similar results in three separate studies. Therefore, the pEY2E1-B construct is up to four times more potent in driving cell-mediated immune responses. We also determined whether CD8+ lymphocytes were responsible for the IFN-γ secretion detected in BalB/C mice immunized with pEY2E1-B. As shown in Fig. 5B, the number of SFU/per million cells was reduced to 127.5 + 11 after CD8+ depletion, indicating that there was about 90% of decrease in the frequencies of IFN-γ producing cells observed by CD8+ T-cell depleted ELISpot. The IFN-γ production induced by pEY2E1-B is mediated mainly by CD8+ T-cells.

In addition, in order to model human T cell immune responses to HLA-A2 presented antigens and identify those antigens, we performed the same ELISpot assay mentioned above using transgenic HLA-A2.1/H2-Dd mice. As shown in Fig 5C, the additive frequency of SFU/per million splenocytes for all four pools in pEY2E1-B immunized transgenic mice was 2362 + 257, while the number of SFU/per million cells in pEK2P-B immunized transgenic mice was only 493 + 57. These results indicated that the pEY2E1-B construct is up to four times more potent in driving cell-mediated immune responses in the transgenic mice. The ELISpot data after

CD8 depletion suggested that the IFN-γ production induced by pEY2E1-B is primarily mediated by CD8+ T-cells (Fig. 5D).

Moreover, we were interested in further detailing the cellular immune responses that were observed in the ELISpot assay. Accordingly, an additional set of ELISpot assay was performed against libraries of peptides spanning the consensus subtype B envelope protein. A complete set of 15-mer peptides overlapped by 11 amino acids, which comprise the subtype B consensus envelope protein, was used to perform this mapping study. The study illustrated that there was no clear dominant epitope induced by the synthetic envelope. However, IFN-γ ELISpot analysis of splenocytes derived from the pEY2E1-B-vaccinated BalB/C mice revealed that there were 18 pools out of 29 pools showing more than 50 spots, while there were only 6 pools in pEK2P-B vaccinated BalB/C mice (Fig. 5E). These results illustrated that there is a significant increase in the breadth and magnitude of cellular immune responses induced by the EY2E1-B immunogen.

Strong cross-reactive cellular immune responses induced by pEY2E1-B. To determine whether the EY2E1-B immunogen could induce broad and cross-reactive cellular immune responses, IFN-γ ELISpot was performed both in BalB/C and HLA-A2 transgenic mice using HIV-1 group M, consensus subtype C, HIV-1 MN (subtype B isolate), HIV-1 C.UY.01.TRA3011 and C.ZA.01.J54Ma (two subtype C isolates) envelope peptides. These assays will further determine if the results observed in Fig. 5A, C and E alone are related to the peptide targets or actually due to the increase in immune breadth. As shown in Fig. 6A, the additive number of SFU/per million splenocytes against four pools of HIV-1 MN envelope peptides in pEY2E1-B vaccinated BalB/C mice was 1855 + 215.8, which was about two times more than those in pEK2P-B immunized BalB/C mice (SFU/per million splenocytes was 700 + 168.2), indicating that pEY2E1-B had stronger cross reactivity than pEK2P-B within subtype B. The numbers of IFN-γ spots in response to stimulation with four HIV group M (Fig. 6B) and subtype C (Fig. 6C) consensus envelope peptides pools in pEY2E1-B immunized BalB/C mice were 1150 + 191.3 and 715 + 116.1, respectively. Compared to the numbers of spots against group M and subtype C peptides which were 635 + 152.3 and 345 + 82.3 in pEK2P-B vaccinated BalB/C mice, these data illustrate that the cross-clade immune responses elicited by pEY2E1-B is approximately 45% stronger than those induced by pEK2P-B in BalB/C mice.

Importantly, we observed much stronger cross reactive cellular immune responses induced by pEY2E1-B in transgenic mice (Fig. 6F-J). The additive number of SFU/per million splenocytes against four pools of HIV-1 MN envelope peptides in pEY2E1-B vaccinated transgenic mice was 1087 + 153, which was about three times more than those in pEK2P-B immunized HLA-A2 mice (SFU/per million splenocytes was 316 + 63) (Fig. 6F), indicating that pEY2E1-B could also elicit stronger cross reactivity than pEK2P-B within subtype B in transgenic mice. The numbers of IFN-γ spots in response to stimulation with four HIV group M (Fig. 6G) and subtype C (Fig. 6H) consensus envelope peptides pools in pEY2E1-B immunized transgenic mice were 2116 + 216 and 893 + 154, respectively. Compared to the numbers of spots against group M and subtype C peptides which were 473 + 50 and 266 + 55 in pEK2P-B vaccinated transgenic mice, these data indicated that the cross-clade immune responses elicited by pEY2E1-B is about three to four times stronger than those induced by pEK2P-B in transgenic mice. Moreover, two subtype C isolate peptide sets that should serve as a stringent control for evaluating breadth and cross-reactivity achieved by other peptide sets were used to further determine the cross-clade C immune responses. Although there were not too many differences of cross reactivity against these two subtype C isolate sets elicited by pEY2E1-B and pEK2P-B in BalB/C mice (Fig. 6D and E), the cross-clade reactivity against these two subtype C isolate sets induced by pEY2E1-B is about three times stronger than those induced by pEK2P-B (Fig. 6I and J). The numbers of spots against C.ZA.01.J54Ma and C.UY.01.TRA3011 peptides were 1080 + 206 and 890 + 150 in pEY2E1-B vaccinated transgenic mice, while the numbers were only 305 + 38 and 310 + 62 in pEK2P-B vaccinated transgenic mice.

Finally, we determined whether there was also an increase in the breadth of cross-reactive cellular immune responses against subtype specific targets induced by the EY2E1-B immunogen by detailing the cellular immune responses against HIV-1 MN observed above both in BalB/C and HLA-A2 transgenic mice. An epitope mapping assay was performed against the library of peptides spanning the subtype B MN envelope protein. The results suggested that there was no clear dominant epitope induced by the synthetic envelope in both mouse strains. However, IFN-γ ELISpot analysis of splenocytes derived from the pEY2E1-B-vaccinated BalB/C mice revealed that there were 14 pools out of 29 pools showing more than 50 spots, while there were only 9 pools in pEK2P-B vaccinated BalB/C mice (Fig. 7A). Similarly, in transgenic mice, there were

18 pools out of 29 pools showing more than 50 spots in pEY2E1-B immunized transgenic mice, while there were only 6 pools in pEK2P-B vaccinated transgenic mice (Fig. 7B). These data indicated that there is a significant increase in the breadth and magnitude of cross reactive cellular immune responses induced by the EY2E1-B immunogen both in BalB/C and HLA-A2 transgenic mice.

DISCUSSION

Worldwide HIV-1 DNA vaccine efforts have been guided by the principle that HIV-specific T-cell responses may provide some contribution to protection from infection or control of replication post-infection. DNA vaccines can impact viral replication although in general they are not as potent in immune induction as attenuated live viral vectors (Almond, N., et al.. 1995. Protection by attenuated simian immunodeficiency virus in macaques against challenge with virus-infected cells. Lancet 345:1342-1344; Berman, P. W., et al. 1996. Protection of MN-rgp120-immunized chimpanzees from heterologous infection with a primary isolate of human immunodeficiency virus type 1. J Infect Dis 173:52-9; Boyer, J., et al. 1997. Protection of chimpanzees from high-dose heterologous HIV-1 challenge by DNA vaccination. Nat Med 3:526-532; Daniel, M. C., et al. 1992. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. Science 258:1938-1941). Strategies aimed at improving the breadth and magnitude of the cellular immune responses are therefore important. The present invention provides a novel antigen using several features of immunogens that have been reported in the literature as separate approaches, but have not been previously assembled together in one vaccine modality. As proof of concept, a synthetic engineered consensus-based envelope immunogen was developed and compared with an optimized primary sequence immunogen for induction of cell-mediated immune responses. Expression data showed that this engineered new envelope gene could be efficiently expressed in mammalian cell lines although the expression levels of these two immunogens were very similar (Fig. 3A). We observed in the immunogenicity studies that the cellular immune responses induced by this functional immunogen exhibited increased diversity and magnitude compared to the primary envelope vaccine. Epitope mapping data obtained in both BalB/C and HLA-A2 transgenic mice demonstrated that this diversity and magnitude improvement was maintained across these haplotypes. To further confirm this finding, we also developed a consensus-based subtype C envelope immunogen and compared it

with a primary subtype C immunogen, again the synthetic consensus-based subtype C envelope immunogen exhibited enhanced diversity and magnitude of cellular immune responses compared to the primary C immunogen (unpublished data).

From the point of view of vaccine design strategy, sequence homology between the vaccine candidate and the infecting or challenging virus may be an important consideration. An effective approach to minimize the degree of sequence dissimilarity between a vaccine strain and contemporary circulating viruses is to create artificial sequences that are "central" to these viruses. One strategy to design such a sequence is to use a consensus sequence derived from the most common amino acid in every position in an alignment. In this study, we developed a consensus-based subtype B envelope vaccine and thought this synthetic immunogen would have higher cross reactivity. Our results did show that there was a diversity of cellular immune responses induced by the pEY2E1-B vaccine. Peptide mapping results in both Balb/c and transgenic mice as well indicated that the EY2E1-B immunogen broadened the immune responses. Moreover, the results of cross-reactive cellular immune responses study indicated that pEY2E1-B could elicit significantly stronger and broader cross-reactive cellular immune responses. Therefore, the artificial consensus envelope immunogens contain more conserved epitopes than found in any individual natural isolate and they induce broader cross-clade CTL responses.

A consensus sequence theoretically has advantages and disadvantages. Since a consensus sequence is generated based on contemporary isolates, it may be genetically closer to current circulating viral strains than any given natural virus isolate. However, since global sequencing is generally conducted with viruses sampled during chronic infections instead of viruses sampled during acute infection, developing a consensus vaccine response on epitopes that for the most part have escaped may be a disadvantage. To minimize this disadvantage, one useful strategy for vaccine design would be to take early transmitter sequences into account. Envelope proteins are among the most difficult HIV proteins to construct artificially because the hypervariable regions in HIV-1 envelope gene evolve by rapid insertion and deletion and not by point mutation. The difference of hypervariable regions in length makes it hard to generate the consensus sequences of these regions. Recently, Gao et al. (Gao, F., Eet al. 2005. Antigenicity and immunogenicity of a synthetic human immunodeficiency virus type 1 group m consensus envelope glycoprotein. J

Virol 79:1154-63) generated a group M consensus envelope sequence, however, the nonconsensus sequences from corresponding regions of a CRF08 BC recombinant strain were used in these variable regions. Studies have indicated that subtype C viruses encoding envelope glycoproteins with shorter V1, V2 and V4 regions are transmitted in recipients with a frequency significantly greater than would be expected by chance. The subtype A envelope sequences from early infection also had significant shorter V1 and V2 loop sequences and fewer potential N-linked glycosylation sites (Chohan, B., D. et al. 2005. Selection for Human Immunodeficiency Virus Type 1 envelope glycosylation variants with shorter V1-V2 loop sequences occurs during transmission of certain genetic subtypes and may impact viral RNA levels. J. Virol. 79:6528-6531). In contrast, recently transmitted subtype B variants didn't have shorter V1 and V2 loops. However, it may be important to note the subtype B infection cases were primarily the result of homosexual transmission or drug injection use. Moreover, studies have suggested that a possible functional consequence of having a compact V1, V2 region is to increase exposure of the CD4 binding domain, and then to enhance susceptibility to neutralization (Edwards, T. G., et al. 2001. Relationships between CD4 independence, neutralization sensitivity, and exposure of a CD4-induced epitope in a Human Immunodeficiency Virus type 1 envelope protein. J. Virol. 75:5230-5239; Kolchinsky, P., et al. 2001. Increased neutralization sensitivity of CD4-independent Human Immnuodeficiency Virus variants. J. Virol. 75:2041-2050; Pickora, C., et al. 2005. Identification of two N-linked glycosylation sites within the core of the Simian Immunodificiency virus glycoprotein whose removal enhances sensitivity to soluble CD4. J. Virol. 79:12575-12583; Puffer, B. A., et al.. 2002. CD4 independent of Simian Immunodeficiency Virus Envs is associated with macrophage tropism, neutralization sensitivity, and attenuated pathogenicity. J. Virol. 76:2595-2605). We shortened the V1 and V2 regions when we generated the subtype B consensus sequence.

The early phase of HIV-1 infection is dominated by non-syncytium-inducing (NSI) viruses, which replicate slowly and use CCR5 as their main coreceptor. Syncytium-inducing (SI) viruses, which emerge in about 50% of infected individuals preceding an accelerated CD4 cell decline and progressive clinical course of infection, use CXCR4 as the main coreceptor. A differential coreceptor usage of HIV variants has been demonstrated for all subtypes. Subtype C viruses appear to be different from most other subtypes because an underrepresentation of

CXCR4 using HIV variants in subtype C has frequently been reported. Therefore, CCR5 utilization should be a very crucial consideration for a vaccine design. Previous reports showed that the V3 region of gp120 plays an important role in coreceptor utilization. Six residues in V3 loop has been identified to be critical for CCR5 interaction: arginine307, lysine314, isoleucine316, arginine322, phenylalanine324 and alanine337. However, based on the sequences of subtype C early transmitters, the residue at position 322 should be glutamine instead of arginine. In summary, based on the previous studies showing residues important for CCR5 utilization and the sequences of early transmitters, we designed the subtype B consensus envelope immunogen that could drive immune responses that may in theory target CCR5 coreceptor utilization.

To maximize potential cross-reactivity, a HIV-1 group M consensus envelope sequence has been created. However, it is possible that subtype-specific envelope consensus vaccines may represent a compromise for the overall sequence similarity of the vaccine antigen relative to circulating viruses at least at the level of cellular immune responses. Studies have shown that there were high rates of selection identified in different regions of subtype B and C envelope proteins. This may be caused by different immune pressure on different regions of the envelope protein in subtype B and C. Therefore, there may be advantages in using a subtype-specific envelope vaccine, as the immune responses to the vaccine and the circulating virus would share antigenic domains. More experiments comparing group M and subtype-specific envelope vaccines are needed to further clarify this issue.

Another important concern about using a consensus sequence is that its sequence may associate polymorphisms in combinations not found in any natural virus, thus potentially resulting in improper protein conformations. Previous studies has indicated that a group M consensus immunogen could fold into native conformation, preserve envelope antigenic epitopes and elicit weak neutralizing antibody response. Based on the facts that the synthetic protein could bind to antibodies 2G12, ID6 and F105, we think that the pEY2E1-B may have somewhat native structural confirmations. Importantly, our data also demonstrated that EY2E1-B immunogen could induce a higher-titer subtype B envelope-specific antibody, indicating this synthetic immunogen may preserve more Class II epitopes as well. More studies in this area will be important.

With the generation of new HIV-1 vaccine strategies, there is also an increasing demand to predict the efficacy of these vaccines in human using preclinical models. In our study, HLA-A2 transgenic mice were used to study the cellular immune responses elicited by the synthetic immunogen. Studies have shown that this transgenic strain is an important preclinical model for design and testing of vaccines for infectious diseases involving optimal stimulation of human CD8+ cytolytic T cells. In this model the results indicated that EY2E1-B could elicit much broader and stronger cellular immune responses compared to EK2P-B, suggesting that this new vaccine may have more potential to induce HLA-A2-restricted cellular responses. Further study of this immunogen in non-human primates are being planned.

Taken together, our results suggest that EY2E1-B could serve as an immunogen that increases both the magnitude and breadth of CTL responses as a DNA vaccine cassette. In more general terms, this construct may be useful in other platforms for induction of stronger and broader cellular immune responses against HIV strains in non-DNA vector approaches.

Example 2 Development of a Novel Engineered HIV-1 Clade C Envelope DNA Vaccine that Enhances Diversity and Breadth of the Elicited Cellular Immune Response

Strong HIV-1 specific CTL responses have an important role in managing viral load during acute and asymptomatic infection. However, recent studies on consensus immunogens have not been able to noticeably demonstrate improved cellular immune responses. Here we test a novel engineered Clade C consensus-based envelope immunogen for improved cellular immune response. The novel vaccine (pEY3E1-C) was created from the HIV-1 Clade C consensus envelope sequence. Several modifications were performed including shortening the highly variable V1 and V2 regions based on early transmitter sequence, retention of the V3 loop for CCR5 utilization, removal of the cytoplasmic tail region from the C-terminus to prevent envelope recycling, and retention of the cleavage site and TMD for proper folding. Also, an IgE leader sequence was added to the N-terminus. This consensus DNA vaccine was also RNA optimized and codon optimized. The cellular immune response was studied in BalB/C mice via ELISpot and epitope mapping assays. When studied as a DNA vaccine, compared to pEK3P-C (derived from a primary isolate of Clade C env), our construct (pEY3E1-C) was more effective at driving a cellular immune response. pEY3E1-C elicited a cellular immune response greater in magnitude than pEK3P-C when stimulated by Consensus Clade C peptides. Additionally, the consensus immunogen elicited an increase in the magnitude of the cellular immune response

when stimulated by two other sets of primary isolate peptides also from Clade C. In addition to augmented magnitude, enhanced breadth of the CTL response was supported by the pEY3E1-C's ability to induce at least 15 out of 29 strongly reactive peptide pools (having more than 50 spots/per million splenocytes), while pEK3P-C only induced 3 out of 29 pools and 9 out of 29 pools with strong reactivity in response to two primary isolate peptide sets, which were selected for their uniqueness and ability to serve as a stringent control for evaluating breadth. Furthermore, pEY3E1-C elicited a stronger Cross-Clade cellular immune response when stimulated with Clade B peptides. The consensus immunogen pEY3E1-C enhances both the magnitude and breadth of CTL responses as a DNA vaccine cassette, suggesting that the potential for consensus immunogens to serve as a component antigen in a HIV vaccine cocktail merits further examination.

With wide genetic diversity, rapid mutation, and recombination of the existing strains, the difficulty of generating an effective vaccine is tremendous. A candidate DNA vaccine derived from an individual isolate may not be able to elicit the cross-reactivity necessary for protection against the diverse circulating strains of HIV-1.

Additionally, it has been reported that DNA vaccines expressing the HIV-1 envelope glycoprotein are not very immunogenic.

We have used a multiphase strategy to increase the potency of the CTL response elicited by the DNA vaccine to possibly provide protection against circulating strains of the virus.

Recent studies have shown that a consensus immunogen may overcome the diversity obstacle created by the rapidly evolving HIV-1 virus.

Derdeyn et al. found that a shorter V1-V4 region is characteristic of early transmitting subtype C virus and our construct has been designed to carry this feature which might be useful in producing a immune response resulting from early transmitted viruses.

Furthermore, the expression levels of our DNA vaccine have been enhanced by codon optimization, RNA optimization, and the addition of an immunoglobulin leader sequence.

HIV-1 specific CTL responses have been shown to be important in controlling viral load during acute and asymptomatic infection and the development of AIDS, thus the following data focuses on the CTL responses elicited by our novel immunogen.

Figure 13 depicts the immunogen design for development of a novel engineered HIV-1 clade C Envelope DNA Vaccine that enhances diversity and breadth of the elicited cellular immune responses.

Figure 14 shows phylogenetic Relationships: Thirty-Six HIV-1 subtype C envelope sequences, EY3E1-C, EK3P-C, two subtype B, one subtype A and one subtype D sequences (outgroup) were included in the phylogenetic analysis. The subtype C envelope sequences representing a broad sample of diversity were from 12 countries.

Table 3 shows the average and range of percent similarity scores between potential envelope vaccine candidates and an alignment of subtype C envelope sequences.

Table 3

	Average % Similarity Scores	Range of % Similarity Scores
pEY3E1-C	85.3	82.7-93.1
pEK3P-C	87.4	83.6-90.2

Three groups of three Balb/C mice were immunized with 100 μg of DNA 3 times with two weeks between immunizations. On the seventh week, spleens were harvested for cellular studies.

As shown in Figure 15 Panels A and B, strong cellular response elicited by pEY3E1-C.

Figure 16 shows strong and broad cellular responses elicited by pEY3E1-C. When stimulated with 29 pools of Consensus C env peptides: pEY3E1-C vaccinated mice elicited more than 50 spots/million splenocytes from 23 pools; pEK3P-C vaccinated mice elicited more than 50 spots/million splenocytes from 2 pools.

Figure 17 Panels A-D show strong cross-reactive cellular responses elicited by pEY3E1-C within the same clade.

Figure 18 Panels A and B show strong and broad cross-reactive cellular responses elicited by pEY3E1-C. Panel A shows data from subtype C (Uruguay) env-Specific IFN-γ ELISpot. When stimulated with 29 pools of Clade C (Uruguay) env peptides: pEY3E1-C vaccinated mice elicited more than 50 spots/million splenocytes from 12 pools; pEK3P-C vaccinated mice elicited more than 50 spots/million splenocytes from 3 pools. Panel B shows data from Subtype C (S. Africa) env-Specific IFN-γ ELISpot. When stimulated with 29 pools of

Clade C (S. Africa) env peptides: pEY3E1-C vaccinated mice elicited more than 50 spots/million splenocytes from 13 pools; pEK3P-C vaccinated mice elicited more than 50 spots/million splenocytes from 5 pools.

Figure 19 Panels A-f show strong cross-reactive cellular responses elicited by pEY3E1-C between clades.

There is a significant increase in the breath and magnitude of cellular immune responses induced by the EOC immunogen. Broader cross-clade reactivity appears as an additional benefit of this immunogen.

Example 3:

Efficacy of a novel engineered HPV-16 DNA vaccine encoding a E6/E7 fusion protein

The immunogen has been designed to be expressed as a polyprotein whereby E6 and E7 sequences are separated by a proteolytic cleavage site. The polyprotein is also expressed with an IgE leader sequence. The polyprotein design includes deletions or mutations in the E6 sequence which are important for p53 binding and degradation and mutations in Rb binding site on the E7 protein. Figure 23 provides an illustration of the immunogen design.

Coding sequences encoding the polyprotein were inserted into the vector pVAX to produce plasmid p1667 Figure 24 shows maps of pVax and p1667.

TC1 tumor cells were immortalized with HPV-16 E7 and transformed with the c-Ha-ras oncogene. These cells express low levels of E7 and are very tumorigenic.

In the immunogenicity study in mice, 3 mice/per group of C57BL6 mice were administered 100 μg DNA/per mouse. Groups included 1) control which were administered pVAX- control vector and 2) test which were administered p1667. Mice were vaccinated on days 0, 14 and 28. On day 35, mice were sacrificed and ELISPOT was performed (Focus on CMI).

The data for cellular immune responses induced by the DNA Immunogen p1667 is shown on Figure 25. HPV16 consensus E6 and E7 peptides (37, 15-mers overlapping by 9 aa) were used in two pools - pool 1: 18 peptides; pool 2: 19 peptides. Panels A and C show data from total spleenocytes. Panels B and D show data from samples with CD8 depletion.

Figure 26 shows results of immunodominant epitope mapping. Two sequences are noted.

In prophylactic experiments in mice, 5 mice/per group of C57BL6 mice were administered 100 μg DNA/per mouse. Groups included 1) naïve (PBS injected), 2) control which were administered pVAX- control vector and 3) test which were administered p1667. Mice were vaccinated on days 0, 14 and 28. On day 35, mice were challenged with TC-1 cells and thereafter tumor size measurements were made. Results are shown in Figure 27. Data from a group in which IL-15 construct was co-administered is also shown.

In tumor regression experiments in mice, 5 mice/per group of C57BL6 mice were administered 100 μg DNA/per mouse. Groups included 1) naïve (PBS injected), 2) control which were administered pVAX- control vector and 3) test which were administered p1667. Mice were challenged with 5 x 104 TC-1 cells at Day 0. Mice were administered DNA vaccine on days 3, 10 and 17. Tumors were measured starting at day 8. Results are shown in Figure 28. Data from a group in which IL-15 construct was co-administered is also shown.

The level of E7 Tetramer positive lymphocytes in spleens was determined. Figure 29 shows the data as the percent E7 Tetramer positive lymphocytes. DNA vaccine p1667 induces the activation of E7-specific CD8+ T cells that are $CD62L^{lo}$ within spleens.

The level of E7 Tetramer positive lymphocytes in tumors was determined. Figure 30 shows the data as the percent E7 Tetramer positive lymphocytes. DNA vaccine p1667 induces the activation of E7-specific CD8+ T cells that are $CD62L^{lo}$ within tumors

A E6/E7 DNA Vaccine protection study in transgenic mice was undertaken. A comparison was made among naive, pVAX, p1667, p1667 + IL-15 and E7/HisB. Data is shown in Figure 31. p1667 and p1667 + IL-15 protected completely.

The data presented herein support the following conclusions. The p1667 construct induces a strong cellular immune response capable of inducing E7-specific CD8+ lymphocytes that mediate the elevated IFN-g responses. We have identified both dominant and novel sub-dominant HPV-16 epitopes against which antigen-specific CTL are generated after administration of the DNA construct. The p1667 construct is capable of preventing tumor growth and causing the regression of tumors in both C57/BL6 and transgenic mice. DNA vaccine p1667 shows great potential for a novel therapeutic strategy to target microscopic HPV-associated cancer.

Example 4

Nucleic acid sequences encoding HIV Env consensus sequences may be administered as DNA vaccines in combination with nucleic acid sequences encoding various other HIV proteins such as Gag, Pol, Gag/Pol, Nef, Vif, and Vpr using for example electoporation technology for intramuscular or intradermal delivery. Multivalent/polyvalent HIV vaccine constructs may provide enhanced immune responsed and be particularly useful. In some embodiments, IL-12 coding sequences are additional provided. U.S. Patent application pubicaton number 20070106062, which is incorporated herein by reference, discloses an HIV Vif DNA vaccine. U.S. Patent application pubicaton number 20040106100, which is incorporated herein by reference, discloses HIV vaccines comprising HIV accessory proteins as well as the sequences of such proteins which may be used to prepare additional vaccine constructs. U.S. Patent Nos. 6,468,982, 5,817,637, and 5,593,972, which are incorporated herein be reference disclose DNA vaccines including HIV gag, HIV pol and HIV gag/pol constructs. Electroporation is described in U.S. Patent No. 7,245,963, which is incorporated by reference. PCT application PCT/US97/19502, which is incorporated herein be reference, discloses IL-12 constructs. U.S. Application Publication No. 20070041941 which is incorporated herein be reference, discloses constructs encoding IL-15.

Example 5

Two groups of macaques were IM immunized three times with optimized plasmid gag and env constructs with or without plasmid IL-12. The same immunization strategy was used for two additional groups but the plasmids were delivered with or without *in vivo* electroporation.

Cellular responses were determined by IFNγ ELISpot after each immunization and five months later for memory responses. Throughout the study humoral responses were evaluated by recombinant p24 and gp160 ELISA. The proliferative capacity of antigen-specific T cells were determined by CFSE staining. Intracellular cytokine staining was done to further characterize the functional characteristics of the induced T-cell response.

Plasmid IL-12 enhanced cellular responses to our optimized constructs. However the use of electroporation to enhance the delivery of plasmids was able to improve both the cellular and humoral response compared to IM immunization with plasmid IL-12. The combination of

plasmid IL-12 and electroporation resulted in the best immune responses, both primary and memory, as measured by a variety of parameters.

Optimized DNA constructs encoding HIV *gag* and *env* in rhesus macaques in the presence or absence of plasmid IL-12 as a DNA adjuvant was compared. IL-12 could substantially increase T cell responses 5-fold in a quantitative ELISpot format resulting in substantially better memory T cell responses. However, EP delivered DNA was more efficient at generating T cell responses and memory that were 2-fold higher compared to the IL-12 IM adjuvanted DNA vaccine. The best responses were observed in the combination arm of EP + IL-12 adjuvant. Memory responses in this arm were 10-fold higher than the IM DNA alone and almost 2-fold higher than EP alone. We also observed 4-fold better immune expansion by CFSE in the EP + IL-12 arm compared to EP alone. The presence of polyfunctional T cells also suggested that the DNA + cytokine + EP arm is most effective.

Materials and Methods

Animals:

Rhesus macaques (*Macaca mulatta*) were housed at BIOQUAL, Inc. (Rockville, MD), in accordance with the standards of the American Association for Accreditation of Laboratory Animal Care. Animals were allowed to acclimate for at least 30 days in quarantine prior to any experimentation.

Immunization:

Five rhesus macaques were immunized at weeks 0, 4, and 11 with 1.0mg of pGag4Y and pEY2E1-B. The DNA at each immunization time point was delivered into two injection sites, one in each quadriceps muscle. Three of the macaques were electroporated following IM injection. Another group of five macaques were immunized at weeks 0, 4, and 8 with 1.0mg of pGag4Y, pEY2E1-B, and WLV104. Of the five animals, two animals received the immunization by IM injection and three animals were electroporated following IM injection. All electroporation procedures were performed using the constant current Cellectra™ device (VGX Immune Therapeutics Division of VGX Pharmaceuticals, The Woodlands, TX). Electroporation conditions were 0.5 Amps, 3 pulses, 52 msec pulse length with 1 sec between pulses. This software-controlled device was designed to measure the tissue resistance immediately prior to

plasmid delivery and generation of constant current square wave pulses, eliminating the risk of delivery outside the muscle tissue and potential plasmid loss.

Blood Collection:

Animals were bled every two weeks for the duration of the study. 10 mL of blood were collected in EDTA tubes. PBMCs were isolated by standard Ficoll-hypaque centrifugation and then resuspended in complete culture medium (RPMI 1640 with 2mM/L L-glutamine supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin, 100μg/mL streptomycin, and 55μM/L β-mercaptoethanol.) RBCs were lysed with ACK lysis buffer (Cambrex Bio Science, East Rutherford, NJ).

Plasmids and plasmid products:

Gag4Y contains an expression cassette encoding for a consensus sequence of the *gag* protein of HIV clades A, B, C, and D with several modifications including: the addition of a kozak sequence, a substituted IgE leader sequence, codon and RNA optimization for expression in mammalian cells (SEQ ID NO:11 discloses HIV Gag consensus sequence.). The *Gag4Y* gene was subcloned into the expression vector, pVax, for further study. pEY-2E1-B contains an expression cassette encoding for a consensus sequence of the envelope of HIV clade B. (SEQ ID NO:3 discloses HIV Env consensus sequence.) WLV104M is a plasmid encoding a rhesus IL-12 gene. Plasmids were produced at Aldevron (Fargo, ND), and re-formulated at VGX Immune Therapeutics (The Woodlands, TX), in sterile water for injection with low molecular weight 0.1% poly-L-glutamate sodium salt

CFSE of Cryo-preserved PBMCs

Cryo-preserved PBMCs were quick-thawed in a 37°C water bath and washed with complete media. Cells were incubated overnight in a 37°C incubator and cell counts were obtained the following day. Cells were pelleted and resuspended in 1 ml CFDA SE (Molecular Probes, Eugene, OR) in PBS (1:2000 dilution). Cells were incubated at 37°C for 10 min. Cells were washed with complete media and resuspended to a concentration of $1x10^6$ cells/100 ul and plated in 96 well round bottom plates with 100 ul of 2 μg/ml recombinant HIV-1 p24 or gp120 (ImmunoDiagnostics, Woburn, MA) plus peptide pools. 5 μg/ml Concavalin A (positive) and complete media (negative) were used as controls. Cultures were incubated for 5 days. Cells were first stained with Vivid dye violet, a live/dead cell marker, for 15 min on ice. Cells were washed

once with PBS. Cells were then stained using anti-human CD3-PE (clone SP34-2) (BD Pharmingen) and anti-human CD4-PerCP (clone L200), anti-human CD8-APC (SK1) for 1 hour at 4°C. Cells were then washed twice with PBS and fixed with 1% paraformaldehyde. Data was collected using a LSRII flow cytometer (BD Biosciences, Franklin Lakes, NJ). Flow cytometry data was analyzed using FlowJo software (Tree Star, Ashland, OR), gating on $CD3^+$ lymphocytes. Thirty to fifty thousand $CD3^+$ lymphocytes were collected per sample.

Enzyme Linked Immunosorbant Assay (ELISA):

Ninety-six well plates were coated overnight with 100ng/well of recombinant HIV-1 IIIB p24 or gp120 (ImmunoDiagnostics) to determine HIV gag and env responses respectively. Plates coated with 100ng/well of bovine serum albumin served as a negative control. Plates were blocked with 3%BSA-PBST for 1 hour at 37°C. Plates were then incubated with four-fold serial serum dilutions for 1 hour at 37°C. Goat anti-monkey IgG horseradish peroxidase conjugated antibody was then added at a 1:10,000 dilution (MP Biomedicals, Aurora, OH) to the plates and incubated for 1 hour at 37°C. Tetramethylbenzidine (R&D systems, Minneapolis, MN) was used to develop the plates and reactions were stopped with 2N H_2SO_4. Optical densities (OD) were then measured.

IgG end-point titers were defined as the reciprocal serum dilution that resulted in OD values that were greater than twice the average OD value of the BSA wells.

Enzyme Linked Immunospot Assay (ELISpot)

Antigen specific responses were determined by subtracting the number of spots in the negative control wells from the wells containing peptides. Results are shown as the mean value (spots/million splenocytes) obtained for triplicate wells.

1. Intracellular Cytokine Staining

Antibody Reagents

Directly conjugated antibodies were obtained from the following: BD Biosciences (San Jose, CA): IL-2 (PE), CD3 (Pacific Blue), IFN-γ (PE-Cy7), and TNF-α (Alexa Fluor 700), CD8 (APC) and CD4 (PerCP).

Cell stimulation and staining

PBMCs were resuspended to 1 x 10^6 cells/100 ul in complete RPMI and plated in 96 well plates with stimulating peptides 100ul of 1:200 dilutions. An unstimulated and positive control

(*Staphylococcus* enterotoxin B, 1 µg/mL; Sigma-Aldrich) was included in each assay. Cells were incubated for 5 hours at 37°C. Following incubation, the cells were washed (PBS) and stained with surface antibodies. The cells were washed and fixed using the Cytofix/Cytoperm kit (BD PharMingen, San Diego, CA) according to instructions. Following fixation, the cells were washed twice in the perm buffer and stained with antibodies against intracellular markers. Following staining, the cells were washed, fixed (PBS containing 1% paraformaldehyde), and stored at 4°C until analysis.

Flow cytometry

Cells were analyzed on a modified LSR II flow cytometer (BD Immunocytometry Systems, San Jose, CA). Fifty thousand CD3+ events were collected per sample. Data analysis was performed using FlowJo version 8.4.1 (TreeStar, San Carlos, CA). Initial gating used a forward scatter area (FSC-A) versus height (FSC-H) plot to remove doublets. The events were subjected to a lymphocyte gate by a FSC-A versus SSC plot. Following this, events are sequentially gated on $CD3^+$, $CD8^+$, and $CD4^-$ events versus IFN-γ to account for down-regulation. Following identification of $CD8^+$ T cells, a gate was made for each respective function using combinations that provided optimal separation. After the gates for each function were created, we used the Boolean gate platform to create the full array of possible combinations, equating to 8 response patterns when testing 3 functions. Data are reported after background correction. Thresholds for positive responses were 10 events or 0.05%.

Statistical Analysis

Data are analyzed using Prism Graphpad software, and is expressed as means ± SEM.

Results

ELISpot Analysis

the induction of the cellular immune response was evaluated after each immunization by IFNγ ELISpot. After a single immunization (Figure 1), the group receiving plasmid DNA by IM injection alone displayed weak cellular responses (74 ± 29 SFU/10^6 PBMCs). Co-immunization with rhesus IL-12 plasmid resulted in a higher response (136 ± 51.4 SFU/10^6 PBMCs). The electroporated (EP) group had an average response that was six times higher than the IM group (482 ± 181 SFU/10^6 PBMCs). The combination of IL-12 co-immunization with EP further doubled the number of IFNγ-producing cells (1030 ± 494 SFU/10^6 PBMCs).

After two immunizations (Figure 1), the IM and IM +IL-12 groups had a modest increase in ELISpot counts (104 ± 67.9 SFU/10^6 PBMCs and 223 ± 76.6 SFU/10^6 PBMCs, respectively). EP group had responses that were almost four fold higher (1924 ± 417 SFU/10^6 PBMCs) than the previous immunization and the EP+IL-12 group had again doubled the number of IFNγ-producing cells (2819 ± 872 SFU/10^6 PBMCs) compared to the EP arm alone.

After the third immunization (Figure 1), the number of antigen specific cells in the EP group was more than a log higher than that of the IM group (5300 ± 3781 and 370 ± 110 SFU/10^6 PBMCs, respectively). The IM+IL-12 group also had a dramatic increase in cellular responses with ELISpot counts that were nearly a log higher than the previous immunization (2042 ± 311 SFU/10^6 PBMCs). As with the other two immunizations, the EP+IL-12 group was the most potent of all the vaccination groups (7228 ± 2227 SFU/10^6 PBMCs).

Induction of cross-reactive envelope responses

A successful HIV vaccine will require the induction of a cross-reactive immune responses in this regard it was interesting to see if EP + IL-12 could improve the magnitude of cross-reactivity to divergent peptide libraries. We compared the cross-reactive CTL responses induced by the *env* antigen using a peptide library from a consensus group M. Cross-reactivity was observed in all groups. However the results displayed the same magnitude differences observed in the subtype B ELISpot analysis (Figure 2). After 3 immunizations, the IM group had the lowest response to the group M envelope peptides (222 ± SEM SFU/10^6 PBMCs). The addition of IL-12 doubled the response (540 ± SEM SFU/10^6 PBMCs). Higher group M envelope responses were induced with EP (830 ± SEM SFU/10^6 PBMCs), which were further enhanced with IL-12 co-injection (1238 ± SEM SFU/10^6 PBMCs).

1. Memory T cell Responses

An important issue is to be able to improve the generation of memory responses with the DNA platform. We performed ELISpot analysis five months after the last DNA vaccination (Figure 3). In the IM groups, the addition of plasmid IL-12 resulted in nearly a 10-fold increase in memory cells (751 ± 11.1 and 78.6 ± 16.9 SFU/10^6 PBMCs). It is clear that IL-12 can positively impact this important T cell phenotype. The number of antigen-specific IFNγ producing cells was substantial in the EP group as well, however the IL-12 adjuvant + EP resulted in the most robust memory response (1231 ± 523.5 and 3795 ± 1336 SFU/10^6 PBMCs

respectively), a response showing that the combined technology drives very strong T cell memory responses.

Humoral immune responses to DNA vaccines

A weakness of IM DNA vaccine technology lies in its inability to induce clear antibody responses in non-human primates and in human clinical studies. We evaluated each group's ability to induce both HIV-1 gag and env specific antibody titers to recombinant p24 and gp160 antigens in an ELISA format. For both antigens, the IM and IM + IL-12 groups did not show significant antibody titers (<1:50 endpoint titer). The electroporated groups exhibited dramatically higher gag antibody titers that were able to bind to recombinant p24. Although both the EP and the EP + IL-12 groups had similar endpoint titers at week 12 (22,400 and 12,800 respectively), the EP + IL-12 group generated a more efficient antibody response. That response appeared earlier in the immunization scheme and rose to the maximum level quickest. The env antibody responses also reflected the results we observed with the gag antigen, albeit with lower endpoint titers.

$CD4^+$ and $CD8^+$ T cell proliferation

Having observed substantial ELISpot responses, we next examined additional parameters of cellular immunity. We examined the ability of gag specific $CD4^+$ and $CD8^+$ T cells to proliferate *in vitro* following peptide stimulation among the different immunization arms. Cryo-preserved samples, collected two weeks after the final immunization, were stimulated and analyzed by CFSE assay. The average $CD4^+$ response increased similar to that observed in the ELISpot assay. By comparison, the CD8 proliferation induction was much more dramatic in magnitude. We observed that IL-12 increased $CD8^+$ T cell proliferation over IM alone and EP was substantially higher. The EP + IL-12 group had the highest percentage of $CD8^+$ cells that were able to proliferate after *in vitro* stimulation (2.51 ± SEM % and 4.88 ± SEM %, respectively). Obvious CD8 T cell proliferation bands were observed in the EP + IL-12 arm, demonstrating the potent proliferative potential of this combined immunization.

Polyfunctional $CD8^+$ T cell responses

Although we have clearly observed the induction of a robust IFNγ effector response following EP and IL-12 co-immunization, we wanted to further characterize the functions of the antigen specific $CD8^+$ T cell responses in the various arms. Samples taken three months

following the final immunization were stimulated with gag peptides and stained for intracellular cytokine production of IFNγ, TNFα and IL-2. Out of all groups, only one animal in the IM + IL-12 and one animal in the EP only group had a detectable IFNγ response. However two out of the three animals in the EP + IL-12 immunized group had gag-specific IFNγ producing $CD8^+$ T cells. The IM + IL-12 responder had a small percentage of polyfunctional cells that stained for all three cytokines as well as a population that had lost its ability to produce IL-2. The EP responder had slightly higher polyfunctional responses that were comprised of four different populations. The most dramatic response was seen in the second EP + IL-12 animal. More than 2% of its $CD8^+$ T cells were able to produce all three cytokines and 2% were able to produce both IFNγ and TNFα. Clearly the number of animals in each group is low and requires additional primate studies to confirm these results, however collectively the trends observed appear clear and encouraging.

Discussion

IL-12 as a DNA vaccine adjuvant improved ELISpot responses several fold over plasmid alone. In addition proliferation was clearly enhanced. The EP group exhibited a higher average response than either IM group alone or the IM + IL-12 arm exhibiting a combined ELISpot response that was 3x higher than the IM + IL-12 group. The best ELISpot responses were observed in the EP + IL-12 arm, which was almost 4x over the IM+IL-12 arm 19x IM alone.

After each immunization the magnitude of the antigen-specific response by IFNγ ELISpot was determined. After a single immunization all of the animals in the EP and EP + IL-12 groups not only had detectable responses, they had averages that were higher than those seen in the IM group after three immunizations. After two immunizations, IFNγ responses in the EP and EP + IL-12 groups were comparable to responses that have been reported in studies using viral vectors. Substantial memory responses were observed in the IM + IL-12 and both EP groups five months after the last immunization.

IM immunization, with or without IL-12, did not result in a significant amount of antibody. Electroporation was able to enhance the humor immune response as reported previously. All of the animals in the electroporated groups seroconverted. Although the EP and the EP + IL-12 groups had similar endpoint titers after three immunizations the kinetics of antibody induction was slightly faster in the EP + IL-12 group.

The proliferative capacity of CD8 T cells appeared to be enhanced with EP and plasmid IL-12. This data supports the memory expansion observed in the ELISpot assay where expansion of antigen specific T cell is likely a result of the enhanced proliferative potential of the EP+ IL-12 arm.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; fragments of SEQ ID NO:1; sequences having at least 90% homology to SEQ ID NO:1; fragments of sequences having at least 90% homology to SEQ ID NO:1; SEQ ID NO:3; fragments of SEQ ID NO:3; sequences having at least 90% homology to SEQ ID NO:3; fragments of sequences having at least 90% homology to SEQ ID NO:3; SEQ ID NO:5; fragments of SEQ ID NO:5; sequences having at least 90% homology to SEQ ID NO:5; fragments of sequences having at least 90% homology to SEQ ID NO:5; SEQ ID NO:7; fragments of SEQ ID NO:7; sequences having at least 90% homology to SEQ ID NO:7; fragments of sequences having at least 90% homology to SEQ ID NO:7; SEQ ID NO:9; fragments of SEQ ID NO:9; sequences having at least 90% homology to SEQ ID NO:9; fragments of sequences having at least 90% homology to SEQ ID NO:9; SEQ ID NO:11; fragments of SEQ ID NO:11; sequences having at least 90% homology to SEQ ID NO:11; fragments of sequences having at least 90% homology to SEQ ID NO:11; SEQ ID NO:22; fragments of SEQ ID NO:22; sequences having at least 90% homology to SEQ ID NO:22; fragments of sequences having at least 90% homology to SEQ ID NO:22; SEQ ID NO:30; fragments of SEQ ID NO:30; sequences having at least 90% homology to SEQ ID NO:30; fragments of sequences having at least 90% homology to SEQ ID NO:30; SEQ ID NO:34; fragments of SEQ ID NO:34; sequences having at least 90% homology to SEQ ID NO:34; fragments of sequences having at least 90% homology to SEQ ID NO:34; SEQ ID NO:36; fragments of SEQ ID NO:36; sequences having at least 90% homology to SEQ ID NO:36; fragments of sequences having at least 90% homology to SEQ ID NO:36; SEQ ID NO:38; fragments of SEQ ID NO:38; sequences having at least 90% homology to SEQ ID NO:38; fragments of sequences having at least 90% homology to SEQ ID NO:38; SEQ ID NO:40; fragments of SEQ ID NO:40; sequences having at least 90% homology to SEQ ID NO:40; fragments of sequences having at least 90% homology to SEQ ID NO:40; SEQ ID NO:42; fragments of SEQ ID NO:42; sequences having at least 90% homology to SEQ ID NO:42; and fragments of sequences having at least 90% homology to SEQ ID NO:42.

2. The nucleic acid molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:22; SEQ ID NO:30; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42.

3. The nucleic acid molecule of claim 1 comprising a sequence having at least 95% homology to a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:22; SEQ ID NO:30; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42.

4. The nucleic acid molecule of claim 1 comprising a sequence having at least 98% homology to a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:22; SEQ ID NO:30; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42.

5 The nucleic acid molecule of claim 1 comprising a sequence having at least 99% homology to a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:22; SEQ ID NO:30; SEQ ID NO:34; SEQ ID NO:36; SEQ ID NO:38; SEQ ID NO:40; and SEQ ID NO:42.

6. The nucleic acid molecule of claim 1 comprising a nucleotide sequence that encodes a protein selected from the group consisting of: SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21. SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:31; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

7. A nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:2; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:2; fragments of nucleotide sequences that encode SEQ ID NO:2; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:2; nucleotide sequences that encode SEQ ID NO:4; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:4; fragments of nucleotide sequences that encodes SEQ ID NO:4; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:4; nucleotide sequences that encode SEQ ID NO:6; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:6; fragments of nucleotide sequences that encode SEQ ID NO:6; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:6; nucleotide sequences that encode SEQ ID NO:8; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:8; fragments of nucleotide sequences that encodes SEQ ID NO:8; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:8; nucleotide sequences that encode SEQ ID NO:10; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:10; fragments of nucleotide sequences that encode SEQ ID NO:10; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:10; nucleotide sequences that encode SEQ ID NO:12; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:12; fragments of nucleotide sequences that encodes SEQ ID NO:12; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:12; nucleotide sequences that encode SEQ ID NO:23; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:23; fragments of nucleotide sequences that encodes SEQ ID NO:23; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:23; nucleotide sequences that encode SEQ ID NO:31; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:31; fragments of nucleotide sequences that encodes SEQ ID NO:31; fragments of nucleotide sequences that encodes an amino acid sequence having at least

90% homology to SEQ ID NO:31; nucleotide sequences that encode SEQ ID NO:35; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:35; fragments of nucleotide sequences that encodes SEQ ID NO:35; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:35; nucleotide sequences that encode SEQ ID NO:37; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:37; fragments of nucleotide sequences that encodes SEQ ID NO:37; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:37; nucleotide sequences that encode SEQ ID NO:39; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:39; fragments of nucleotide sequences that encodes SEQ ID NO:39; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:39; nucleotide sequences that encode SEQ ID NO:41; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:41; fragments of nucleotide sequences that encodes SEQ ID NO:41; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:41; nucleotide sequences that encode SEQ ID NO:43; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:43; fragments of nucleotide sequences that encodes SEQ ID NO:43; and fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:43.

8. The nucleic acid molecule of claim 7 comprising a nucleotide sequence that encodes a protein selected from the group consisting of: SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21. SEQ ID NO:23; SEQ ID NO:31; SEQ ID NO:35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

9. The nucleic acid molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:1; fragments of SEQ ID NO:1; sequences having at least 90% homology to SEQ ID NO:1; fragments of sequences having at least 90% homology to SEQ ID NO:1; SEQ ID NO:3; fragments of SEQ ID NO:3; sequences having at least 90% homology to SEQ ID NO:3; fragments of sequences having at least 90% homology to SEQ ID NO:3; SEQ ID

NO:5; fragments of SEQ ID NO:5; sequences having at least 90% homology to SEQ ID NO:5; fragments of sequences having at least 90% homology to SEQ ID NO:5; SEQ ID NO:7; fragments of SEQ ID NO:7; sequences having at least 90% homology to SEQ ID NO:7; fragments of sequences having at least 90% homology to SEQ ID NO:7; SEQ ID NO:9; fragments of SEQ ID NO:9; sequences having at least 90% homology to SEQ ID NO:9; fragments of sequences having at least 90% homology to SEQ ID NO:9; SEQ ID NO:11; fragments of SEQ ID NO:11; sequences having at least 90% homology to SEQ ID NO:11; and fragments of sequences having at least 90% homology to SEQ ID NO:11.

10. The nucleic acid molecule of claim 7 comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:2; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:2; fragments of nucleotide sequences that encode SEQ ID NO:2; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:2; nucleotide sequences that encode SEQ ID NO:4; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:4; fragments of nucleotide sequences that encodes SEQ ID NO:4; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:4; nucleotide sequences that encode SEQ ID NO:6; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:6; fragments of nucleotide sequences that encode SEQ ID NO:6; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:6; nucleotide sequences that encode SEQ ID NO:8; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:8; fragments of nucleotide sequences that encodes SEQ ID NO:8; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:8; nucleotide sequences that encode SEQ ID NO:10; nucleotide sequences that encode an amino acid sequences having at least 90% homology to SEQ ID NO:10; fragments of nucleotide sequences that encode SEQ ID NO:10; fragments of a nucleotide sequence that encode an amino acid sequence having at least 90% homology to SEQ ID NO:10; nucleotide sequences that encode SEQ ID NO:12; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID

NO:12; fragments of nucleotide sequences that encodes SEQ ID NO:12; and fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:12;

11. The nucleic acid molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:22; fragments of SEQ ID NO:22; sequences having at least 90% homology to SEQ ID NO:22; and fragments of sequences having at least 90% homology to SEQ ID NO:22.

12. The nucleic acid molecule of claim 7 comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:23; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:23; and fragments of nucleotide sequences that encodes SEQ ID NO:23; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:23.

13. The nucleic acid molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:30; fragments of SEQ ID NO:30; sequences having at least 90% homology to SEQ ID NO:30; and fragments of sequences having at least 90% homology to SEQ ID NO:30.

14. The nucleic acid molecule of claim 7 comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:31; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:31; and fragments of nucleotide sequences that encodes SEQ ID NO:31; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:31.

15. The nucleic acid molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:34; fragments of SEQ ID NO:34; sequences having at least

90% homology to SEQ ID NO:34; and fragments of sequences having at least 90% homology to SEQ ID NO:34.

16. The nucleic acid molecule of claim 7 comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:35; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:35; and fragments of nucleotide sequences that encodes SEQ ID NO:35; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:35.

17. The nucleic acid molecule of claim 1 comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:36; fragments of SEQ ID NO:36; sequences having at least 90% homology to SEQ ID NO:36; and fragments of sequences having at least 90% homology to SEQ ID NO:36; SEQ ID NO:38; fragments of SEQ ID NO:38; sequences having at least 90% homology to SEQ ID NO:38; fragments of sequences having at least 90% homology to SEQ ID NO:38; SEQ ID NO:40; fragments of SEQ ID NO:40; sequences having at least 90% homology to SEQ ID NO:40; fragments of sequences having at least 90% homology to SEQ ID NO:40; SEQ ID NO:42; fragments of SEQ ID NO:42; sequences having at least 90% homology to SEQ ID NO:42; and fragments of sequences having at least 90% homology to SEQ ID NO:42.

18. The nucleic acid molecule of claim 7 comprising a nucleotide sequence selected from the group consisting of: nucleotide sequences that encode SEQ ID NO:37; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:37; fragments of nucleotide sequences that encodes SEQ ID NO:37; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:37; nucleotide sequences that encode SEQ ID NO:39; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:39; fragments of nucleotide sequences that encodes SEQ ID NO:39; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:39; nucleotide sequences that encode SEQ ID NO:41; nucleotide sequences that encodes an amino acid sequences having at least 90%

homology to SEQ ID NO:41; fragments of nucleotide sequences that encodes SEQ ID NO:41; fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:41; nucleotide sequences that encode SEQ ID NO:43; nucleotide sequences that encodes an amino acid sequences having at least 90% homology to SEQ ID NO:43; and fragments of nucleotide sequences that encodes SEQ ID NO:43; and fragments of nucleotide sequences that encodes an amino acid sequence having at least 90% homology to SEQ ID NO:43.

19. The nucleic acid molecule of any of claims 1-18 wherein said molecule is a plasmid.

20. A pharmaceutical composition comprising a nucleic acid molecule of any of claims 1-19.

21. An injectable pharmaceutical composition comprising a nucleic acid molecule of any of claims 1-19.

22. A recombinant vaccine comprising a nucleic acid molecule of any of claims 1-18.

23. The recombinant vaccine of claim 22 wherein said recombinant vaccine is a recombinant vaccinia vaccine.

24. A live attenuated pathogen comprising a nucleic acid molecule of any of claims 1-18.

25. A protein comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:2, sequences having at least 90% homology to SEQ ID NO:2; fragments of SEQ ID NO:2; fragments of sequences having at least 90% homology to SEQ ID NO:2; SEQ ID NO:4, sequences having at least 90% homology to SEQ ID NO:4; fragments of SEQ ID NO:; fragments of sequences having at least 90% homology to SEQ ID NO:4; SEQ ID NO:6, sequences having at least 90% homology to SEQ ID NO:6; fragments of SEQ ID NO:6; fragments of sequences having at least 90% homology to SEQ ID NO:6; SEQ ID NO:8, sequences having at least 90% homology to SEQ ID NO:8; fragments of SEQ ID NO:8; fragments of sequences having at least

90% homology to SEQ ID NO:8; SEQ ID NO:10, sequences having at least 90% homology to SEQ ID NO:10; fragments of SEQ ID NO:10; fragments of sequences having at least 90% homology to SEQ ID NO:10; SEQ ID NO:12, sequences having at least 90% homology to SEQ ID NO:12; fragments of SEQ ID NO:12; fragments of sequences having at least 90% homology to SEQ ID NO:12; SEQ ID NO:23, sequences having at least 90% homology to SEQ ID NO:23; fragments of SEQ ID NO:23; fragments of sequences having at least 90% homology to SEQ ID NO:23; SEQ ID NO:31, sequences having at least 90% homology to SEQ ID NO:31; fragments of SEQ ID NO:31; fragments of sequences having at least 90% homology to SEQ ID NO:31; SEQ ID NO:35, sequences having at least 90% homology to SEQ ID NO:35; fragments of SEQ ID NO:35; fragments of sequences having at least 90% homology to SEQ ID NO:35; SEQ ID NO:37, sequences having at least 90% homology to SEQ ID NO:37; fragments of SEQ ID NO:37; fragments of sequences having at least 90% homology to SEQ ID NO:37; SEQ ID NO:39, sequences having at least 90% homology to SEQ ID NO:39; fragments of SEQ ID NO:39; fragments of sequences having at least 90% homology to SEQ ID NO:39; SEQ ID NO:41, sequences having at least 90% homology to SEQ ID NO:41; fragments of SEQ ID NO:41; fragments of sequences having at least 90% homology to SEQ ID NO:41; SEQ ID NO:43, sequences having at least 90% homology to SEQ ID NO:43; fragments of SEQ ID NO:43; and fragments of sequences having at least 90% homology to SEQ ID NO:43.

26 The protein of claim 25 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; and SEQ ID NO:12; SEQ ID NO:23; SEQ ID NO:31; SEQ ID NO 35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

27 The protein of claim 25 comprising a sequences having at least 95% homology to an amino acid sequence selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; and SEQ ID NO:12; SEQ ID NO:23; SEQ ID NO:31; SEQ ID NO 35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

28. The protein of claim 25 comprising a sequences having at least 98% homology to an amino acid sequence selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; and SEQ ID NO:12; SEQ ID NO:23; SEQ ID NO:31; SEQ ID NO 35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

29. The protein of claim 25 comprising a sequences having at least 99% homology to an amino acid sequence selected from the group consisting of: SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; and SEQ ID NO:12; SEQ ID NO:23; SEQ ID NO:31; SEQ ID NO 35; SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

30. The protein of claim 25 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:2, sequences having at least 90% homology to SEQ ID NO:2; fragments of SEQ ID NO:2; fragments of sequences having at least 90% homology to SEQ ID NO:2; SEQ ID NO:4, sequences having at least 90% homology to SEQ ID NO:4; fragments of SEQ ID NO:; fragments of sequences having at least 90% homology to SEQ ID NO:4; SEQ ID NO:6, sequences having at least 90% homology to SEQ ID NO:6; fragments of SEQ ID NO:6; fragments of sequences having at least 90% homology to SEQ ID NO:6; SEQ ID NO:8, sequences having at least 90% homology to SEQ ID NO:8; fragments of SEQ ID NO:8; fragments of sequences having at least 90% homology to SEQ ID NO:8; SEQ ID NO:10, sequences having at least 90% homology to SEQ ID NO:10; fragments of SEQ ID NO:10; fragments of sequences having at least 90% homology to SEQ ID NO:10; SEQ ID NO:12, sequences having at least 90% homology to SEQ ID NO:12; fragments of SEQ ID NO:12; and fragments of sequences having at least 90% homology to SEQ ID NO:12.

31. The protein of claim 30 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:16; SEQ ID NO:17; SEQ ID NO:18; SEQ ID NO:19; SEQ ID NO:20 and SEQ ID NO:21.

32. The protein of claim 25 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:23, sequences having at least 90% homology to SEQ ID NO:23;

fragments of SEQ ID NO:23; and fragments of sequences having at least 90% homology to SEQ ID NO:23.

33. The protein of claim 32 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; and SEQ ID NO:27.

34. The protein of claim 25 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:31, sequences having at least 90% homology to SEQ ID NO:31; fragments of SEQ ID NO:31; and fragments of sequences having at least 90% homology to SEQ ID NO:31.

35. The protein of claim 34 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:31; SEQ ID NO:32; and SEQ ID NO:33.

36. The protein of claim 25 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:35, sequences having at least 90% homology to SEQ ID NO:35; fragments of SEQ ID NO:35; and fragments of sequences having at least 90% homology to SEQ ID NO:35.

37. The protein of claim 34 comprising an amino acid sequence SEQ ID NO:35.

38. The protein of claim 25 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:37, sequences having at least 90% homology to SEQ ID NO:37; fragments of SEQ ID NO:37; fragments of sequences having at least 90% homology to SEQ ID NO:37; SEQ ID NO:39, sequences having at least 90% homology to SEQ ID NO:39; fragments of SEQ ID NO:39; fragments of sequences having at least 90% homology to SEQ ID NO:39; SEQ ID NO:41, sequences having at least 90% homology to SEQ ID NO:41; fragments of SEQ ID NO:41; fragments of sequences having at least 90% homology to SEQ ID NO:41; SEQ ID

NO:43, sequences having at least 90% homology to SEQ ID NO:43; fragments of SEQ ID NO:43; and fragments of sequences having at least 90% homology to SEQ ID NO:43.

39. The protein of claim 38 comprising an amino acid sequence selected from the group consisting of: SEQ ID NO:37; SEQ ID NO:39; SEQ ID NO:41; and SEQ ID NO:43.

40. A pharmaceutical composition comprising a protein of any of claims 25-39.

41. An injectable pharmaceutical composition comprising a protein any of claims 25-39.

42. A recombinant vaccine comprising a protein of any of claims 25-39.

43. The recombinant vaccine of claim 42 wherein said recombinant vaccine is a recombinant vaccinia vaccine.

44. A live attenuated pathogen comprising a protein of any of claims 25-39.

45. A method of inducing an immune response in an individual against HIV comprising administering to said individual a composition comprising a nucleic acid molecule of claim 9 or 10 or a protein of claim 30 or 31.

46. A method of inducing an immune response in an individual against HPV comprising administering to said individual a composition comprising a nucleic acid molecule of claim 11 or 12 or a protein of claim 32 or 33.

47. A method of inducing an immune response in an individual against HCV comprising administering to said individual a composition comprising a nucleic acid molecule of claim 13 or 14 or a protein of claim 34 or 35.

48. A method of inducing an immune response in an individual against hTERT comprising administering to said individual a composition comprising a nucleic acid molecule of claim 15 or 16 or a protein of claim 36 or 37.

49. A method of inducing an immune response in an individual against influenza comprising administering to said individual a composition comprising a nucleic acid molecule of claim 17 or 18 or a protein of claim 38 or 39.

Figure 1

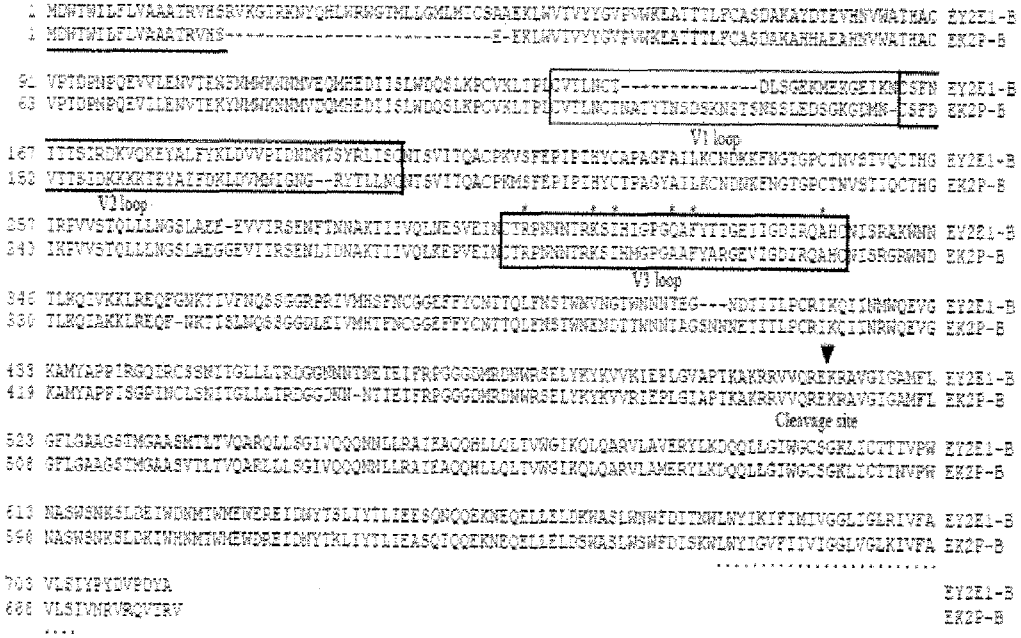


Figure 2

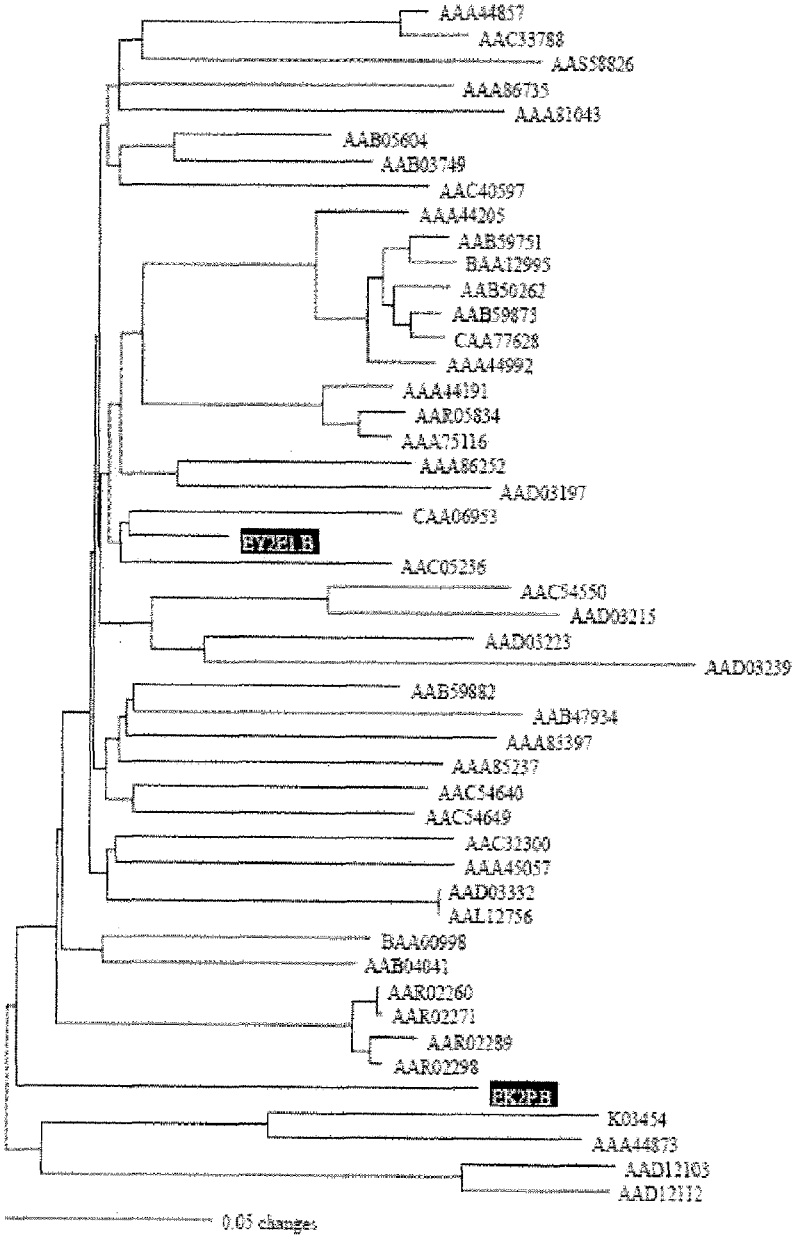
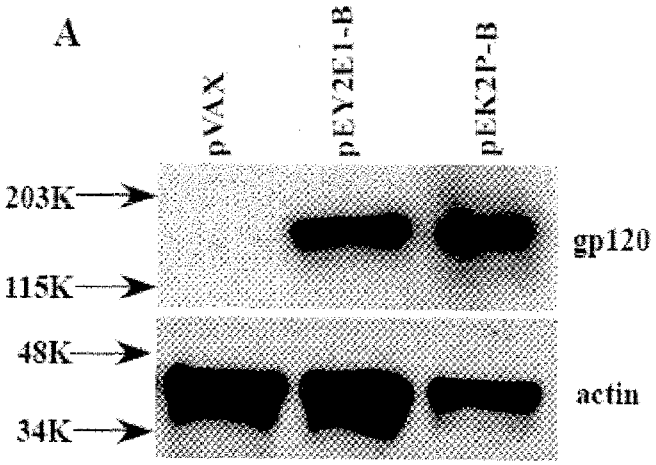


Figure 3, Panels A and B



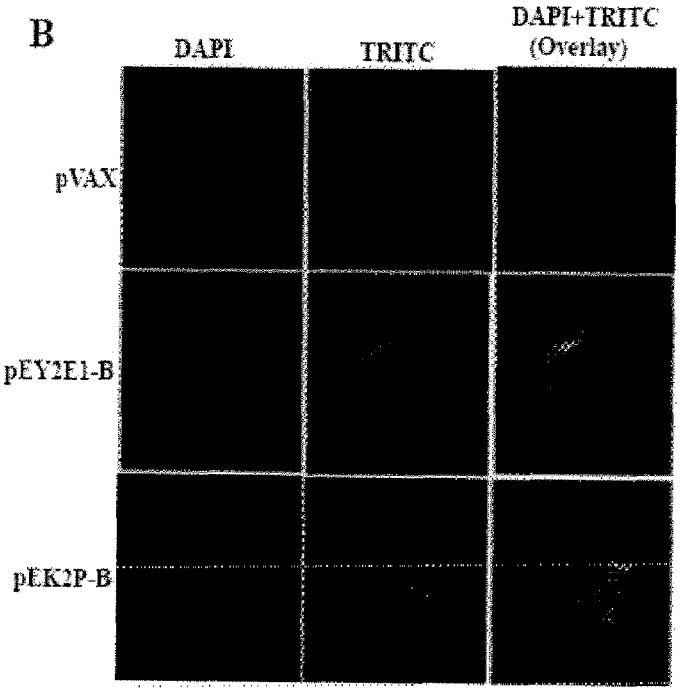
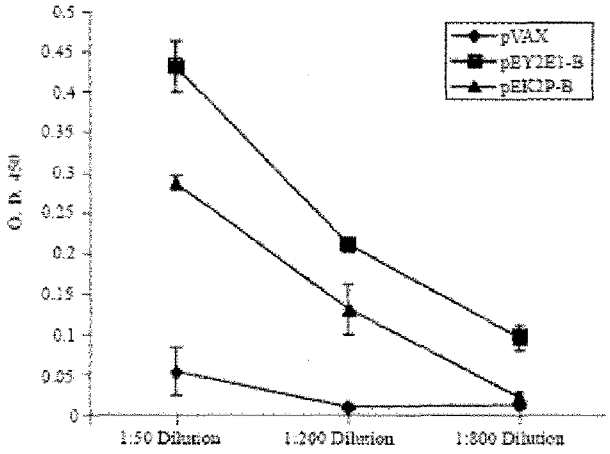


Figure 4
Panel A



Panel B

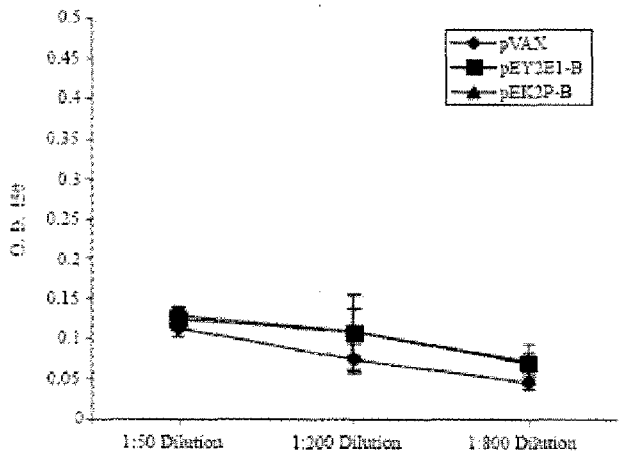


Figure 4
Panel C

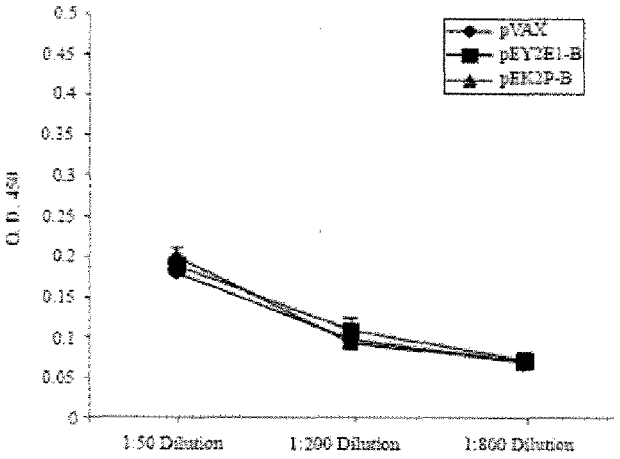


Figure 5
Panel A

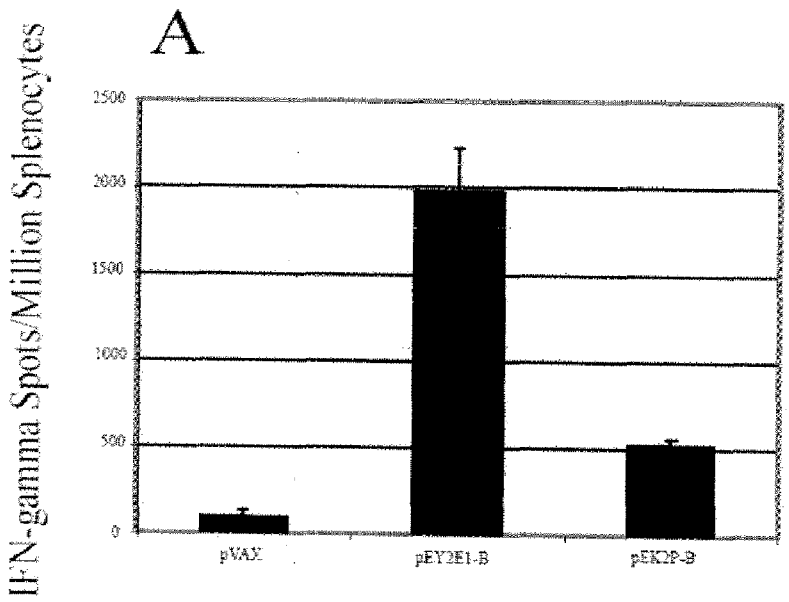


Figure 5
Panel B

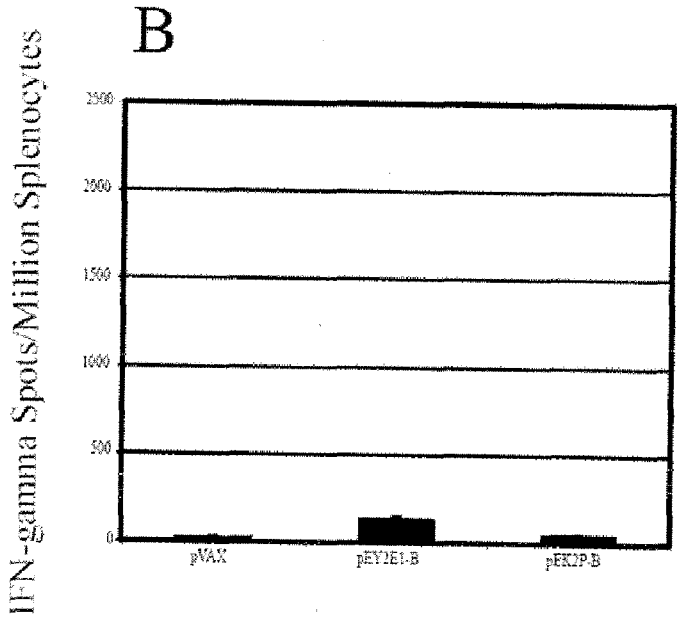


Figure 5
Panel C

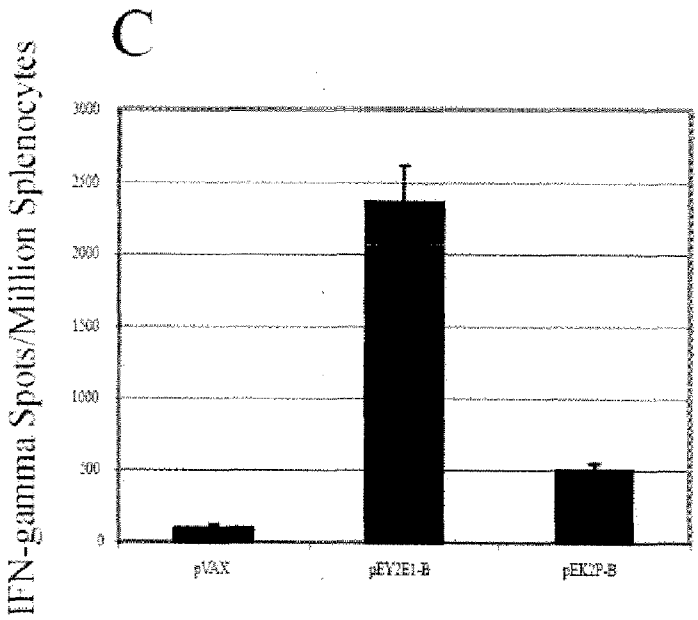


Figure 5
Panel D

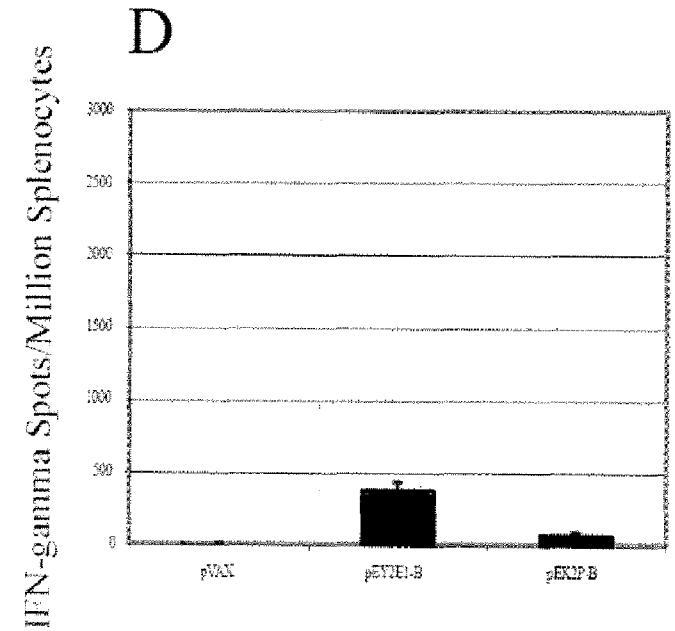


Figure 5
Panel E

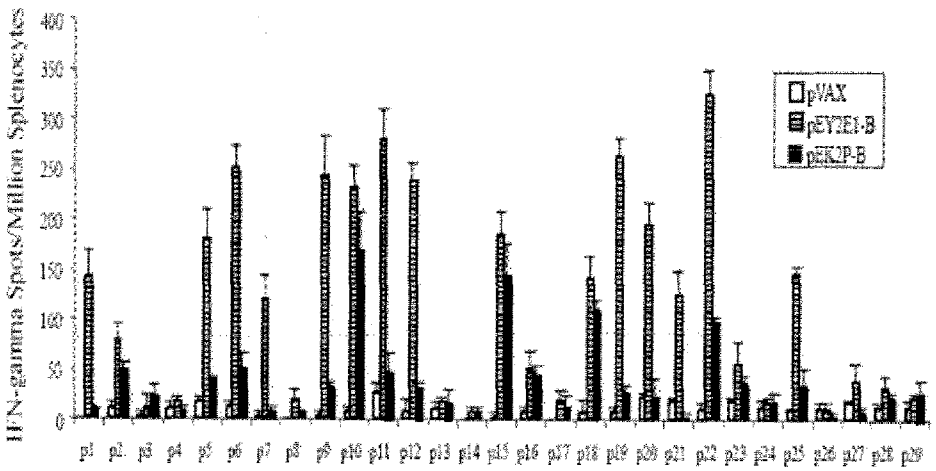


Figure 6
Panels A-D

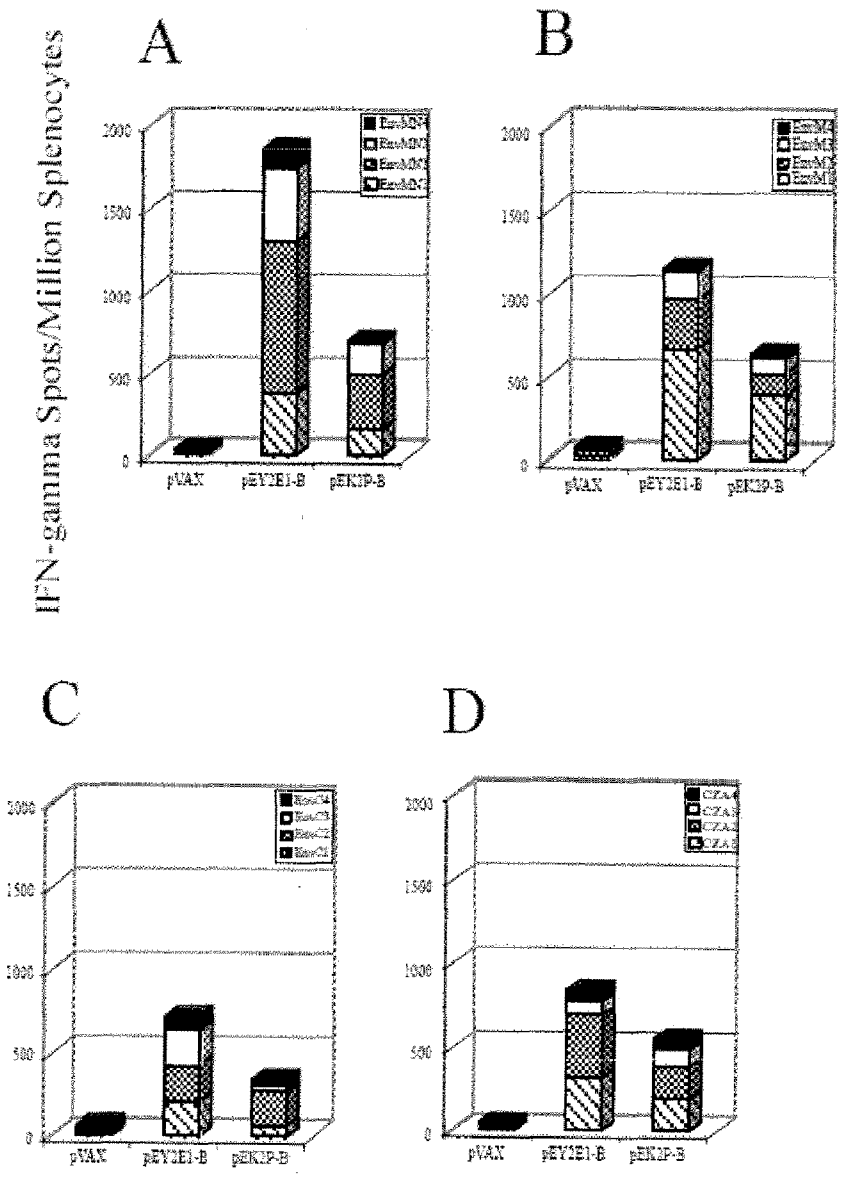


Figure 6
Panel E-H

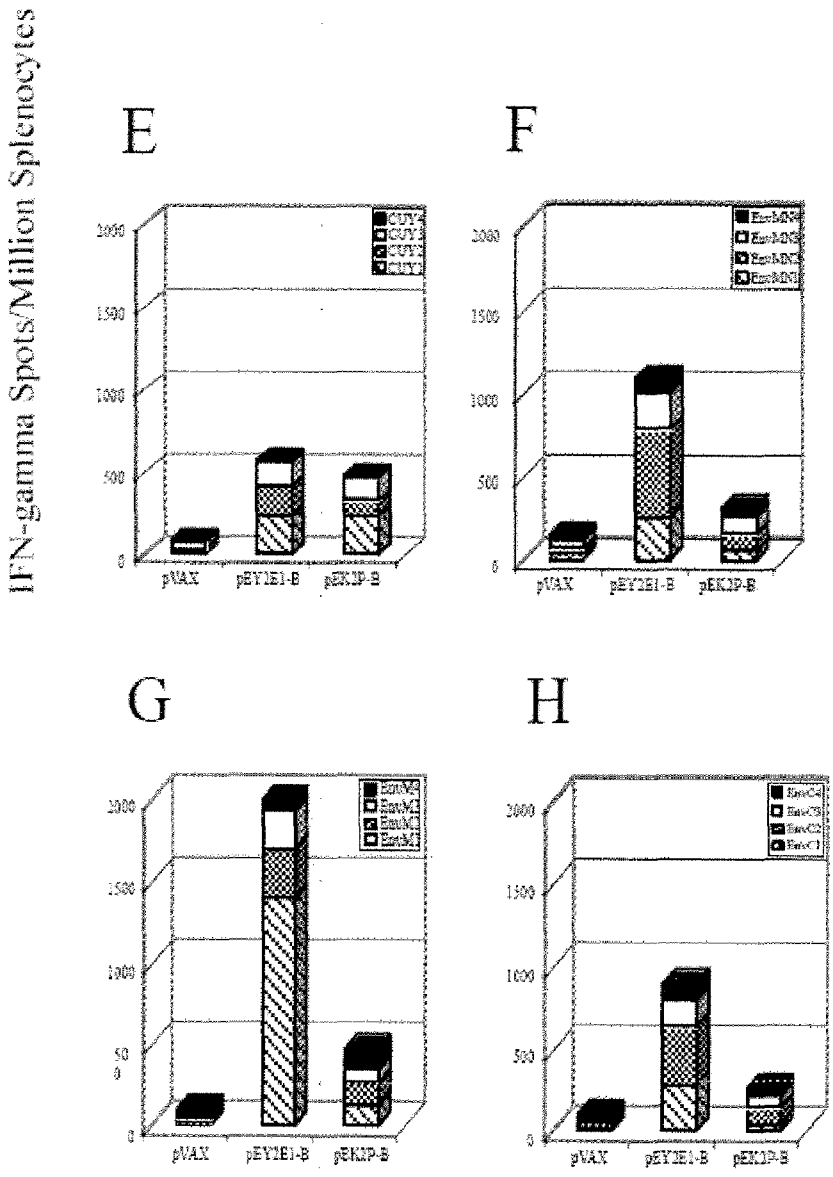


Figure 6
Panels I and J

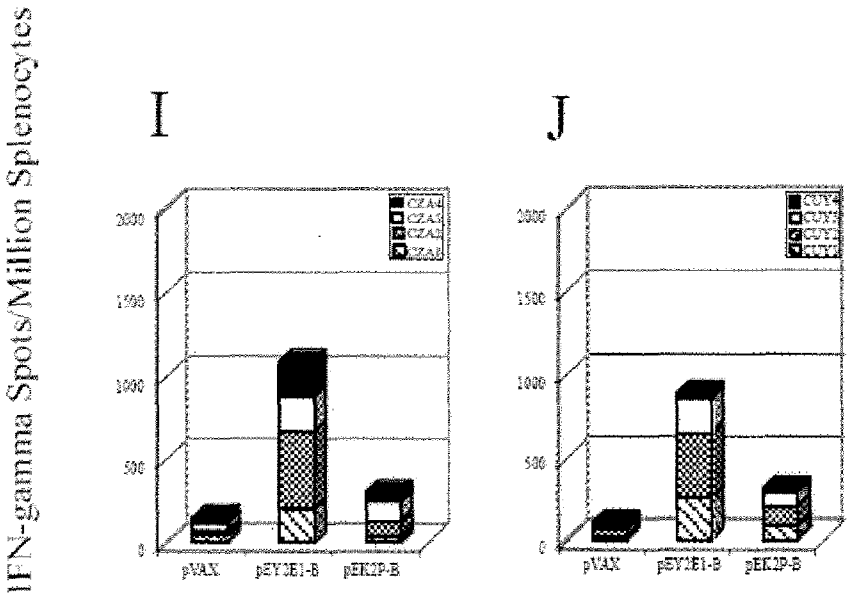


Figure 7
Panel A

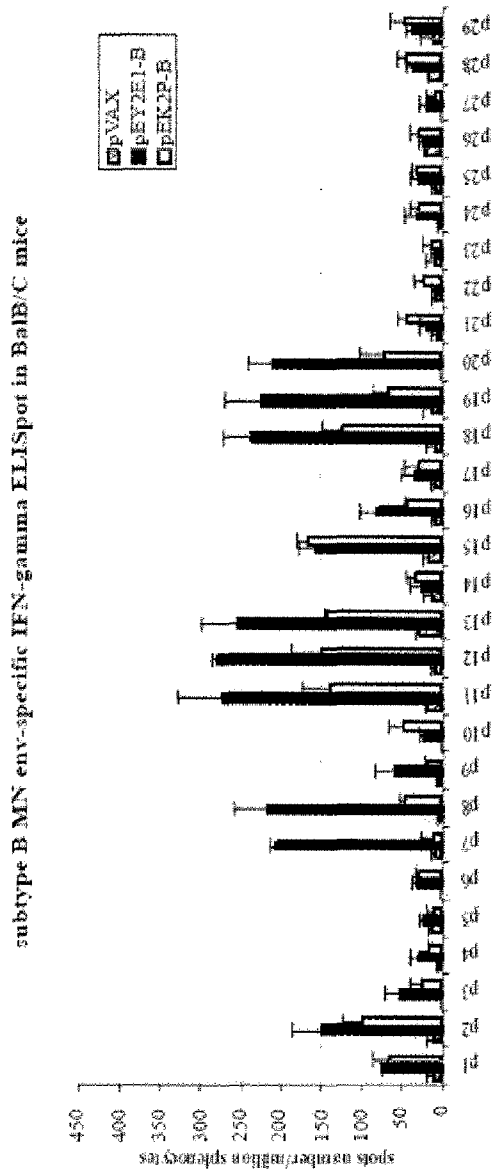


Figure 7
Panel B

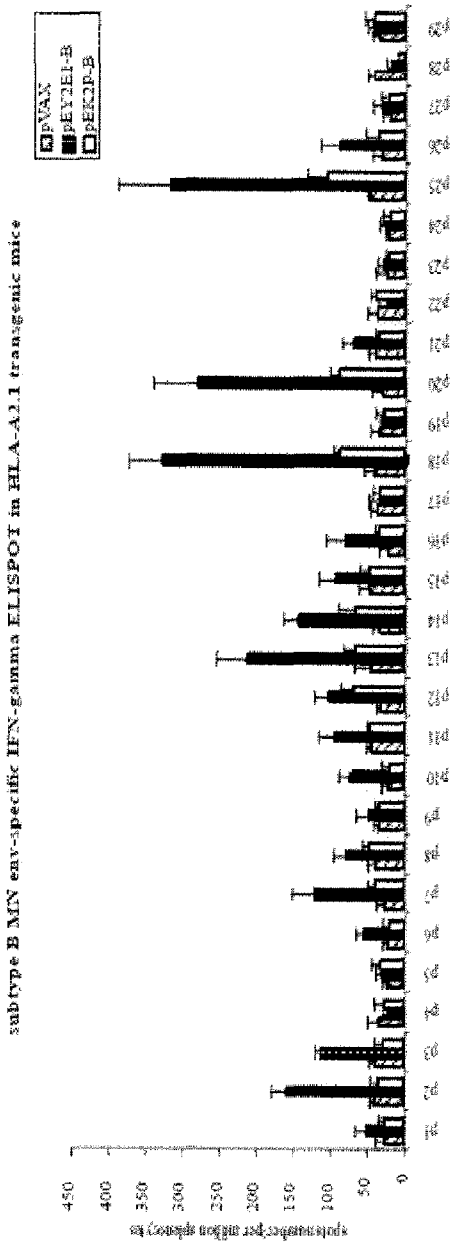


Figure 8

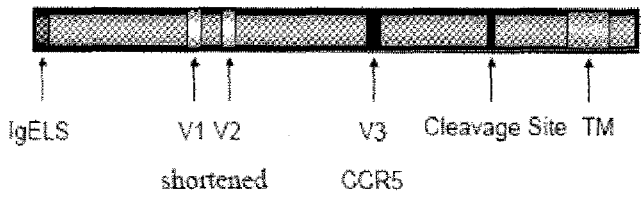


Figure 9

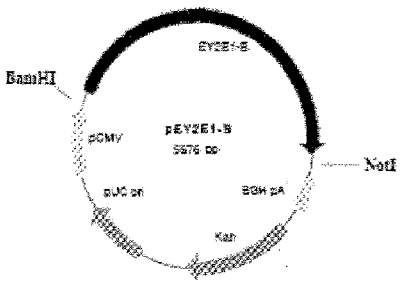
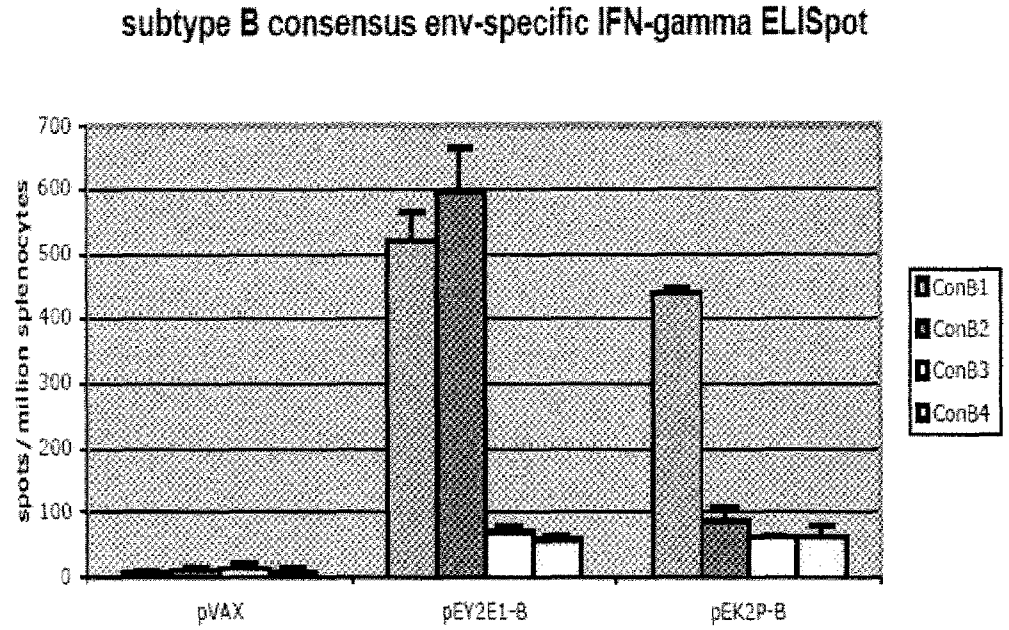


Figure 10
Panel A



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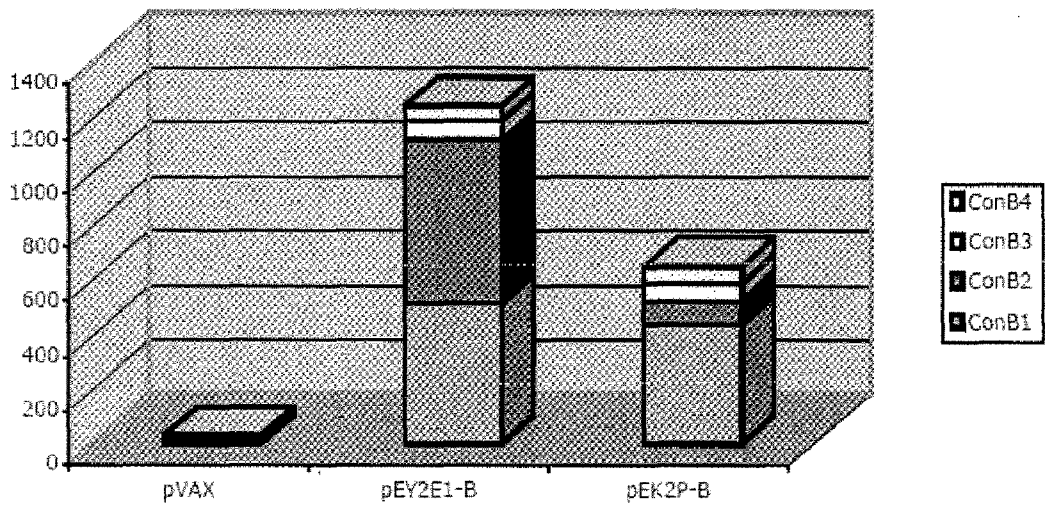
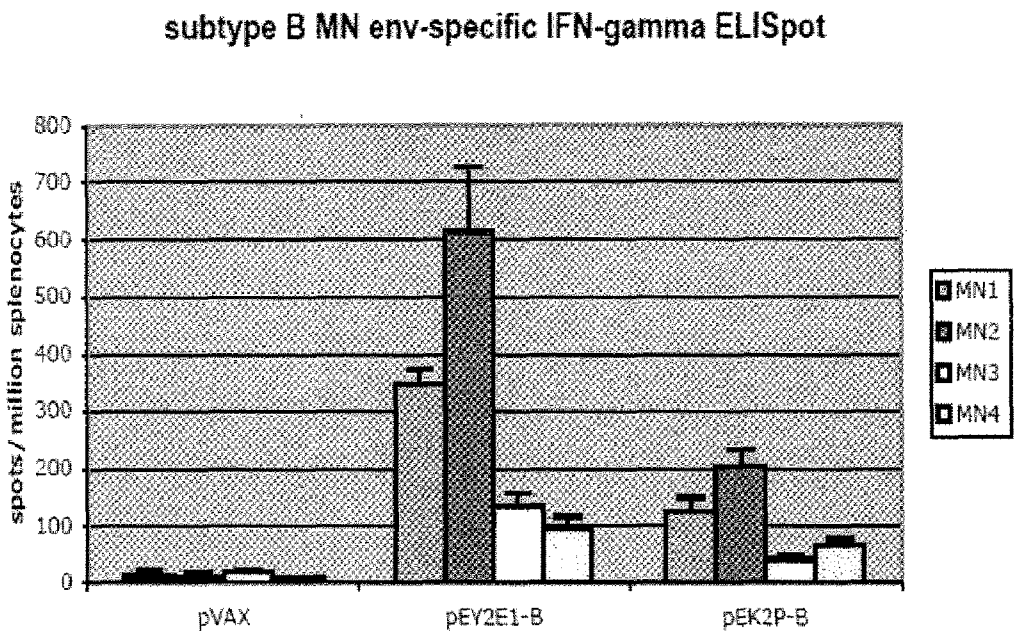


Figure 11
Panel A



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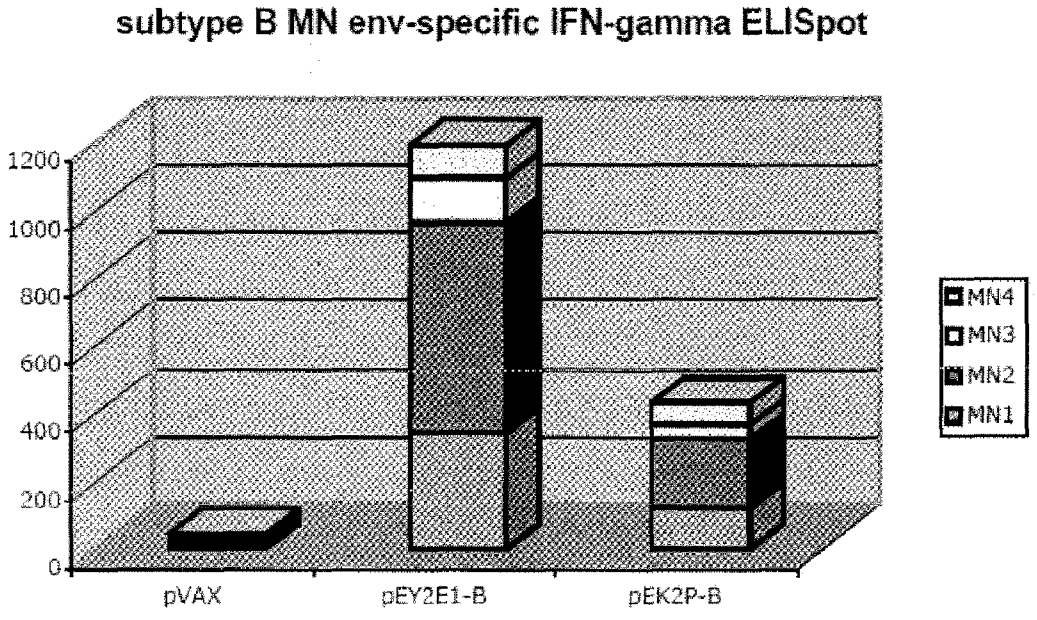
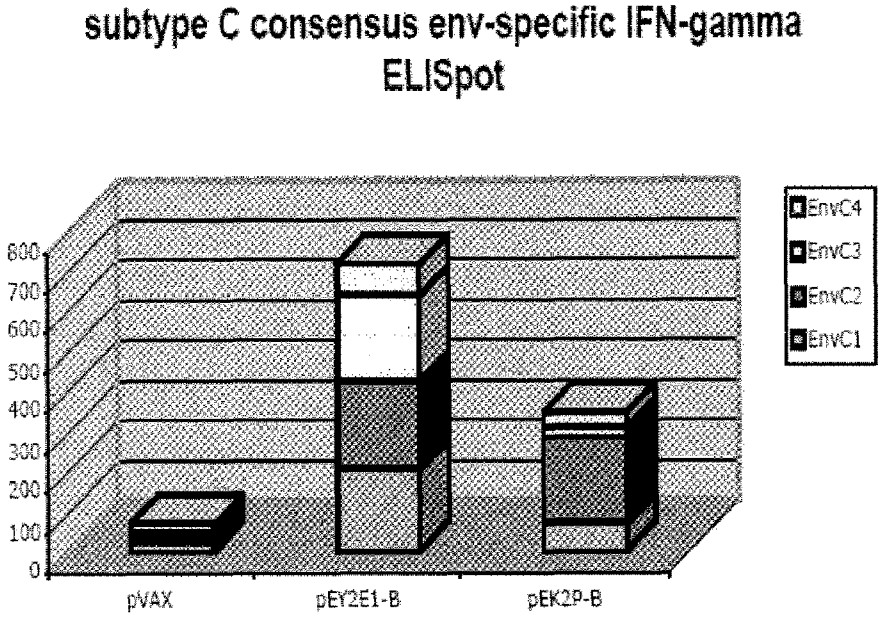


Figure 12
Panel A



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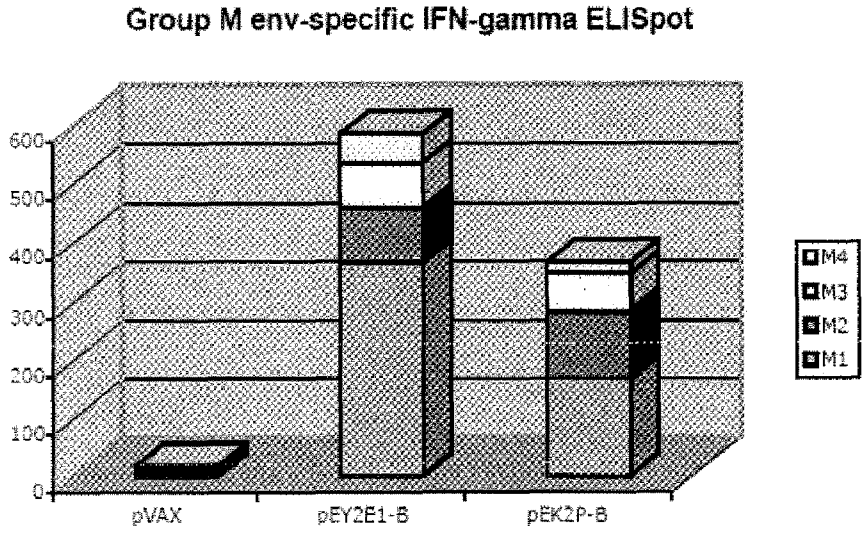
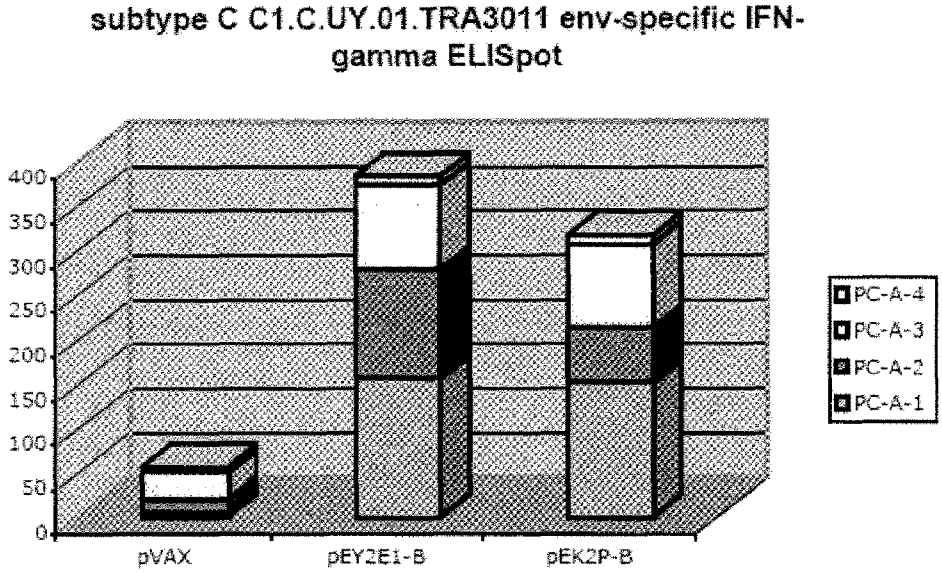


Figure 12
Panel C



Panel D

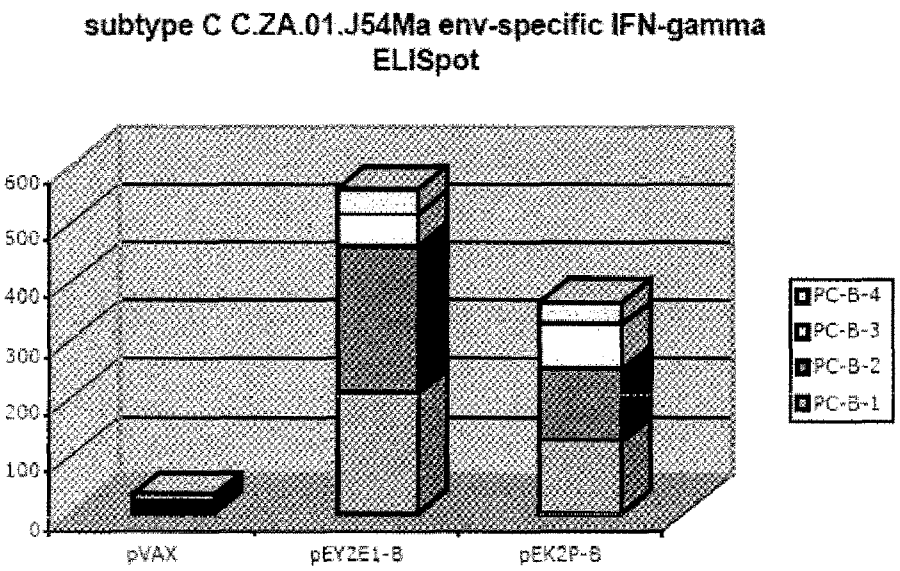


Figure 13

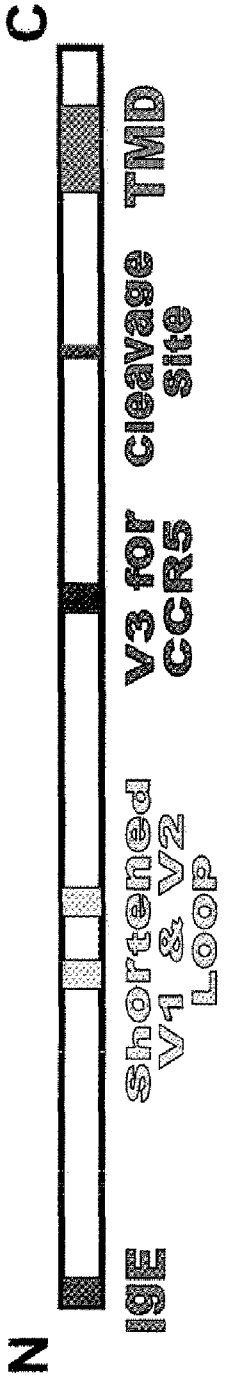


Figure 14

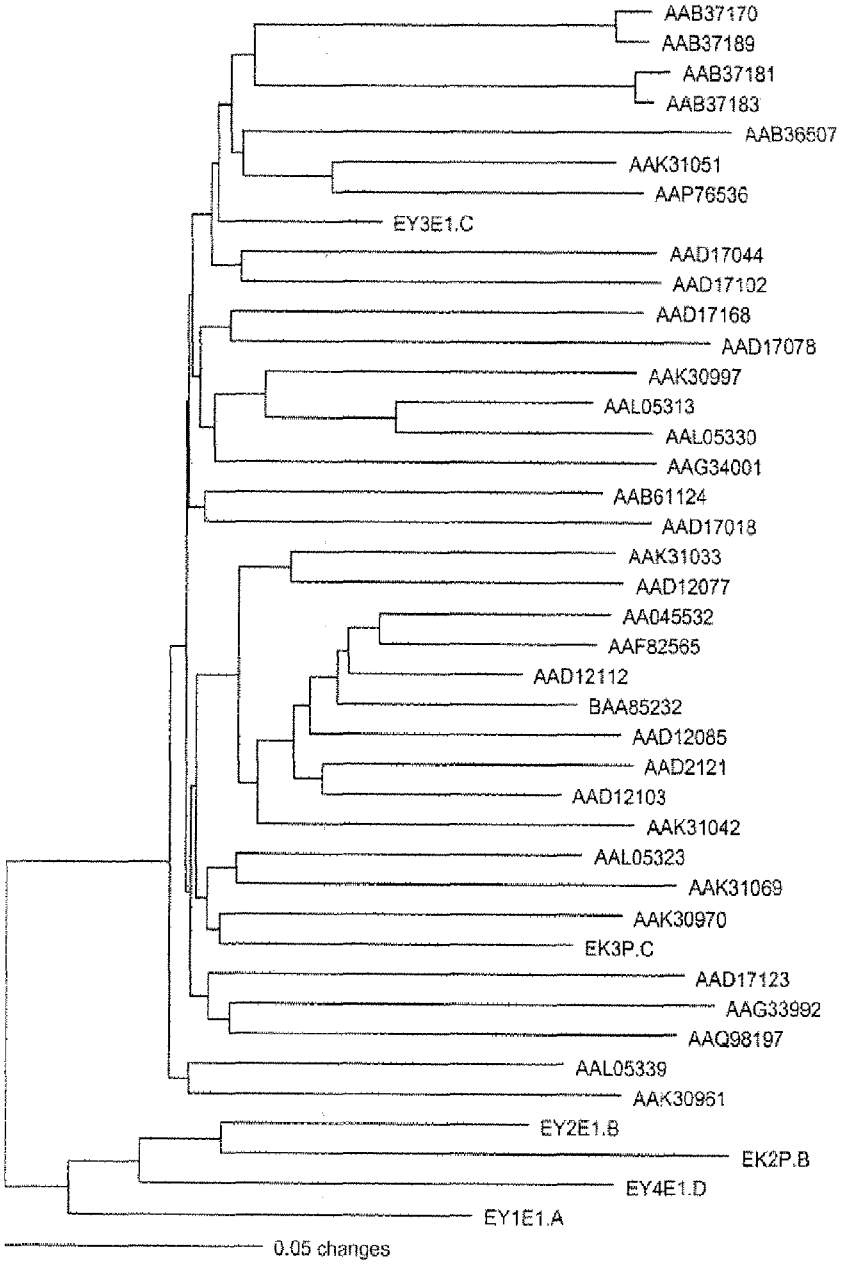
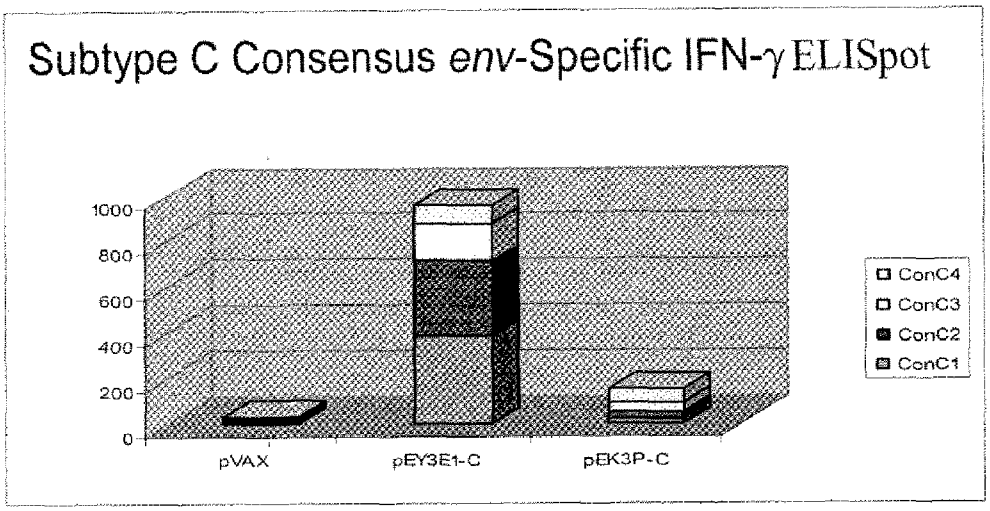


Figure 15

Panel A



Panel B

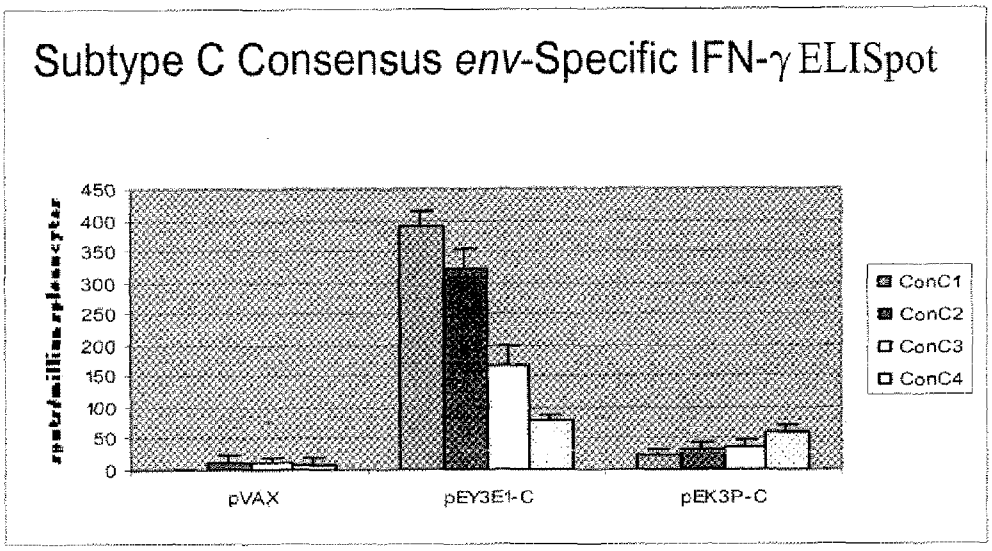


Figure 16

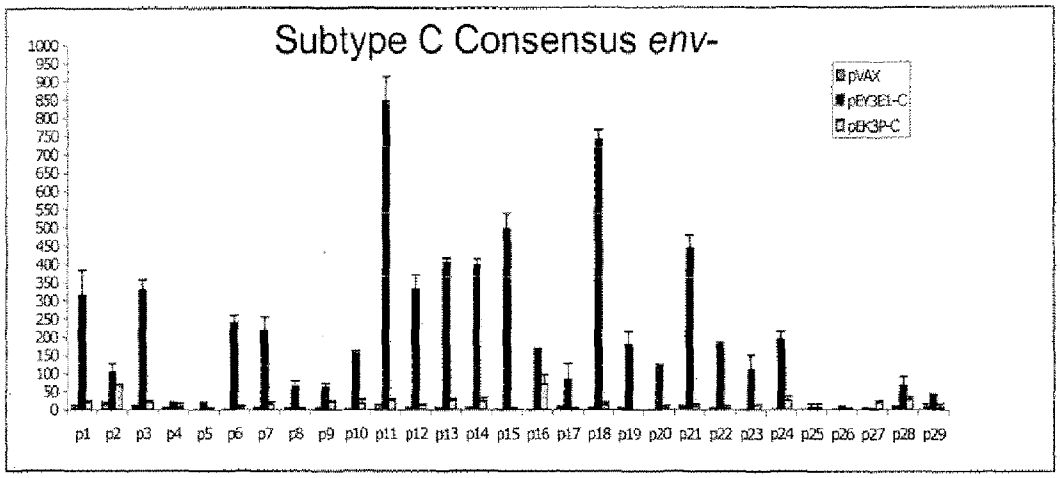
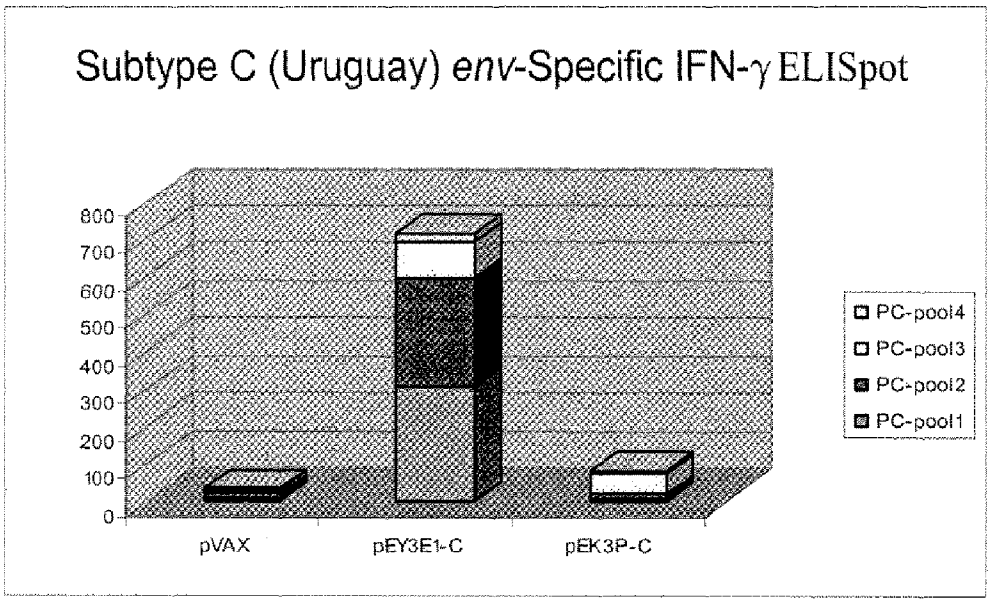


Figure 17
Panel A



Panel B

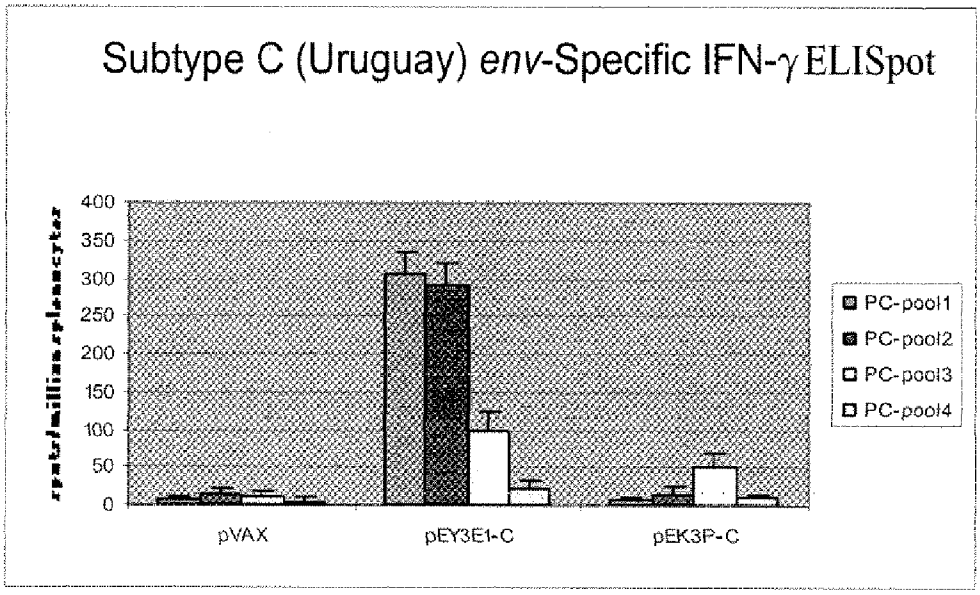
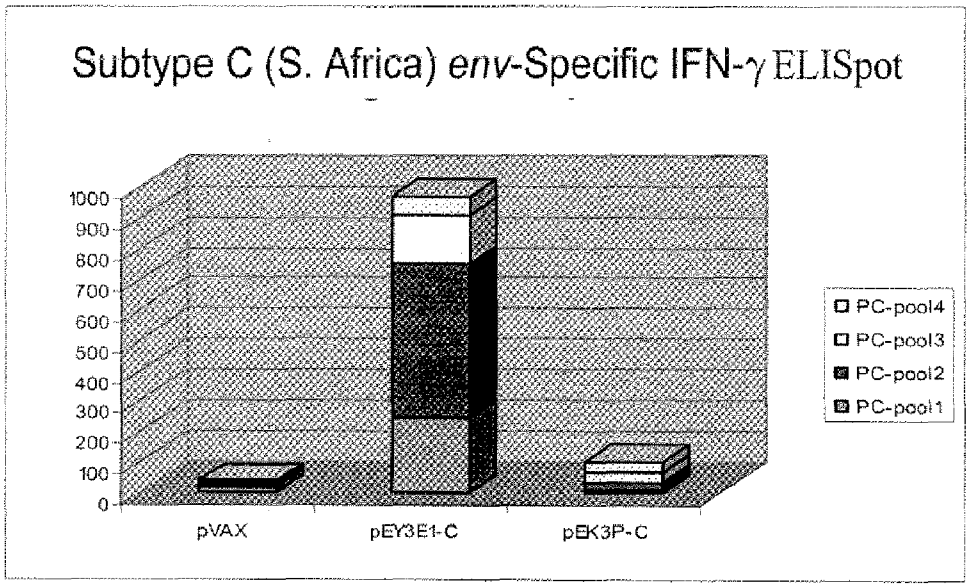


Figure 17
Panel C



Panel D

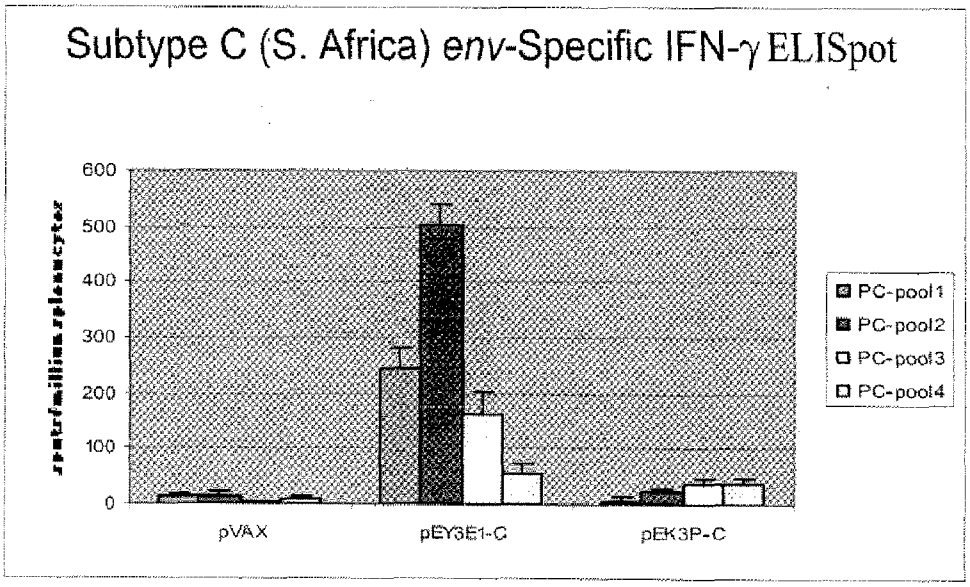
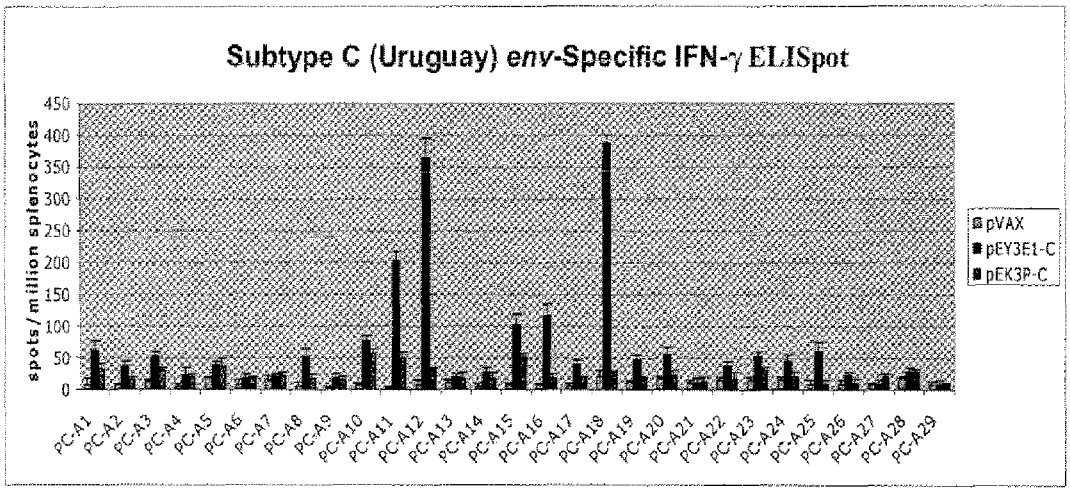


Figure 18
Panel A



Panel B

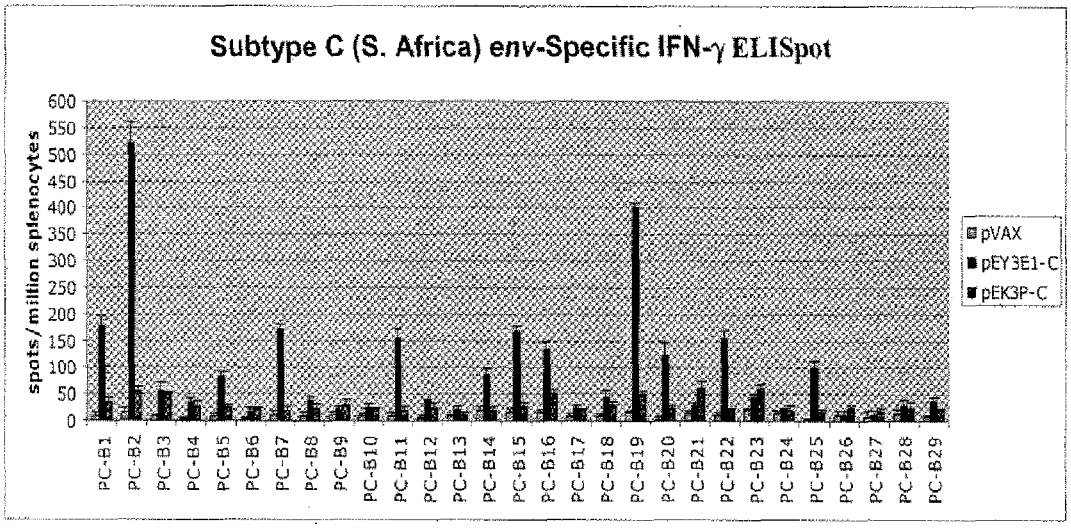
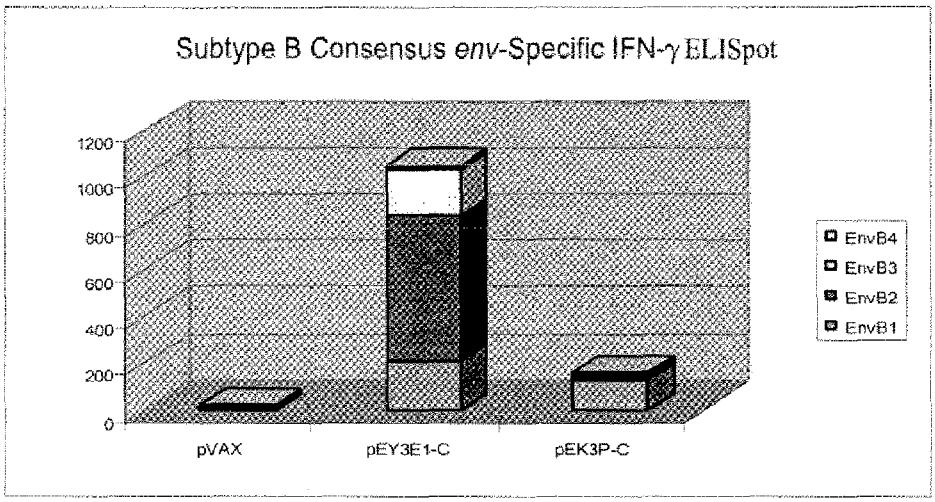


Figure 19
Panel A



Panel B

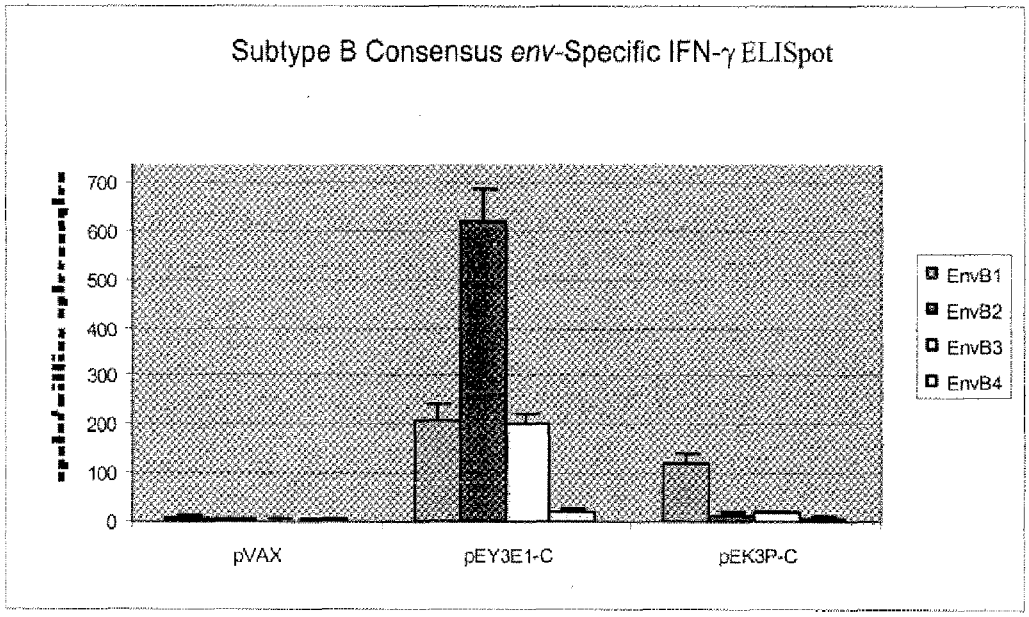
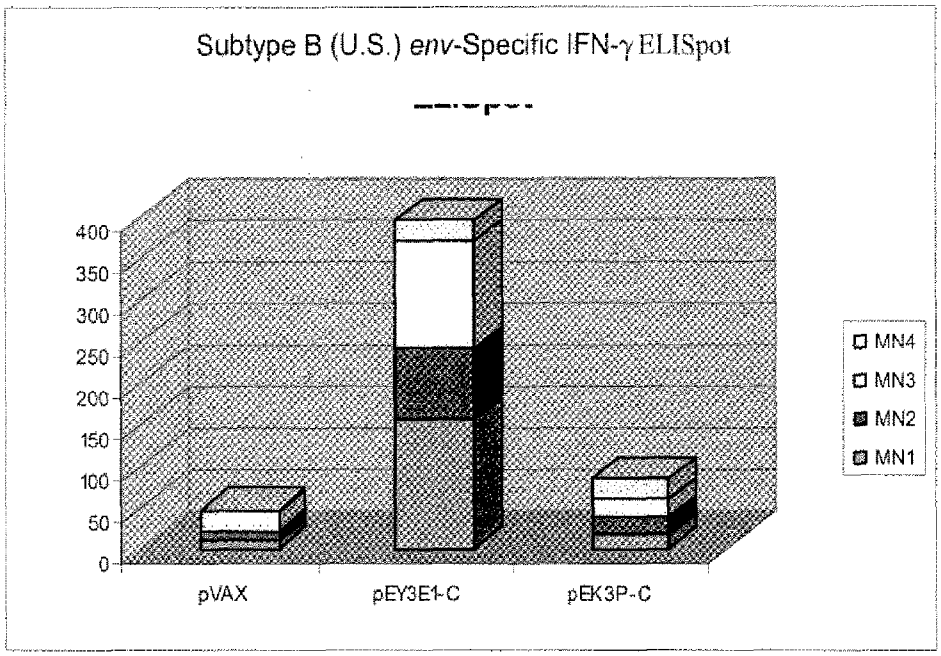


Figure 19
Panel C



Panel D

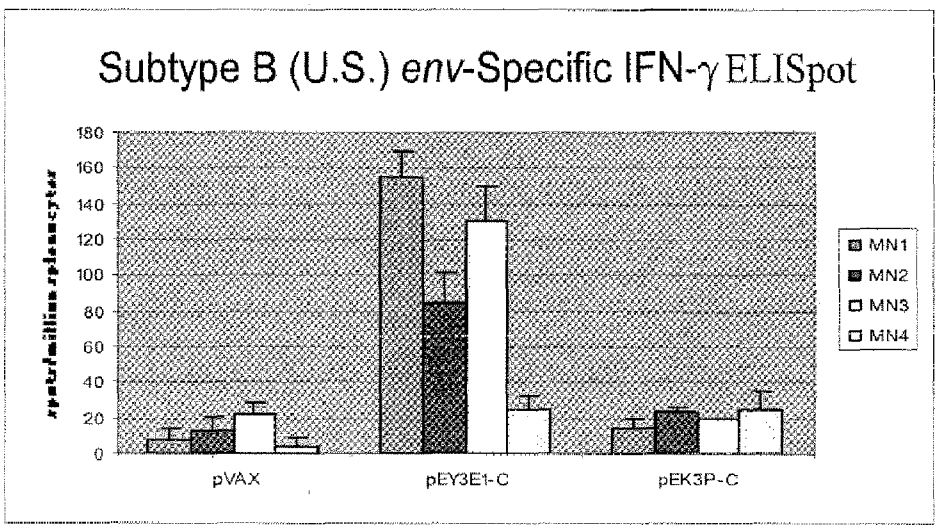
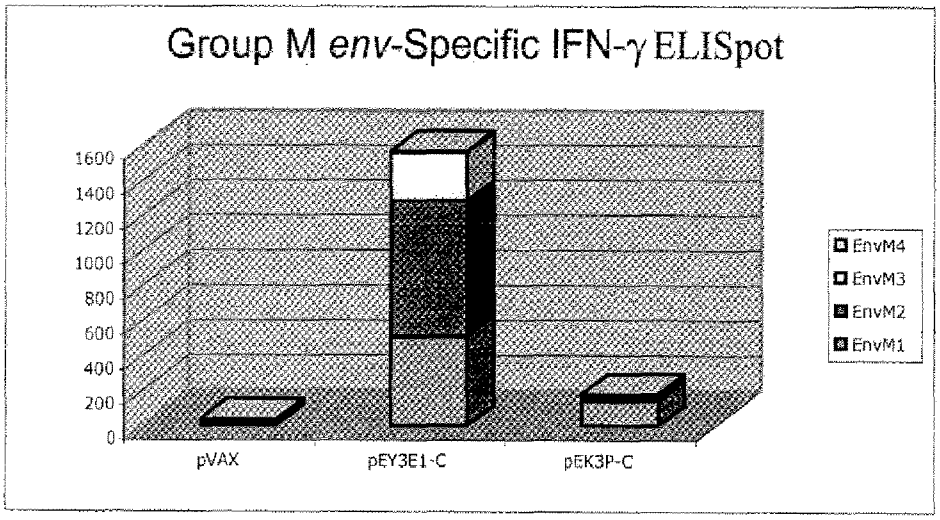


Figure 19
Panel E



Panel F

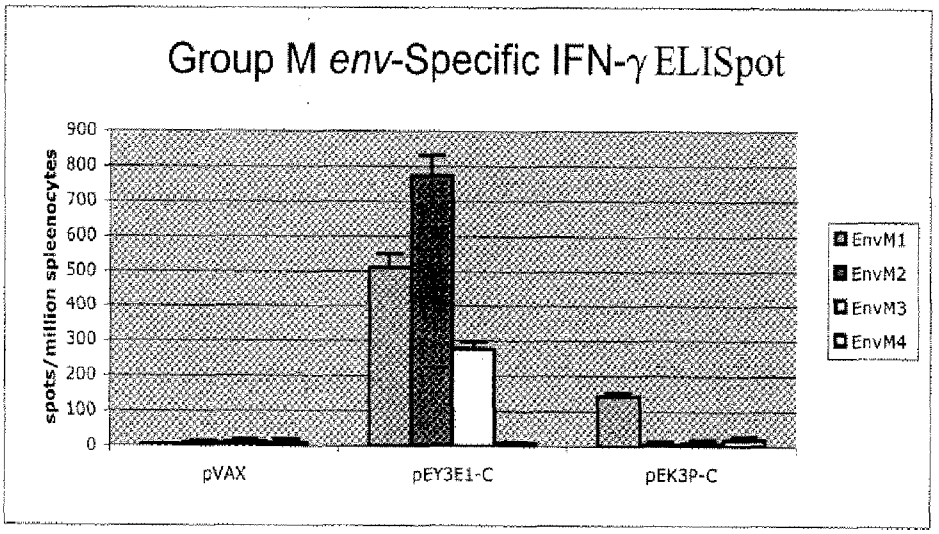
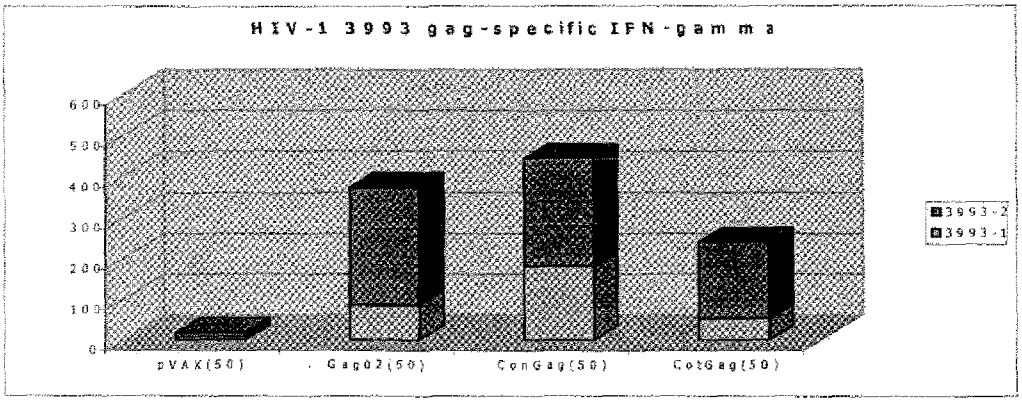
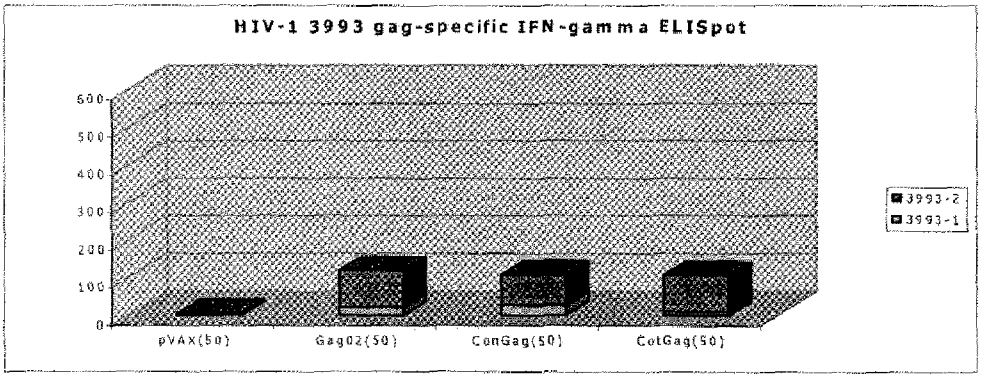


Figure 20
Panel A



Panel B



Panel C

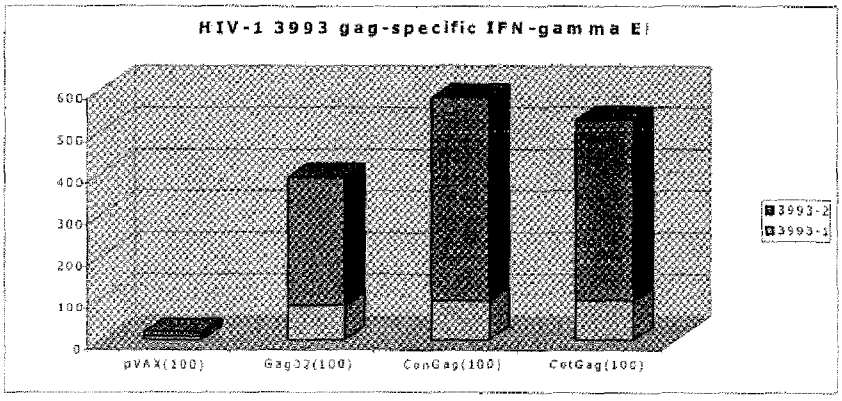
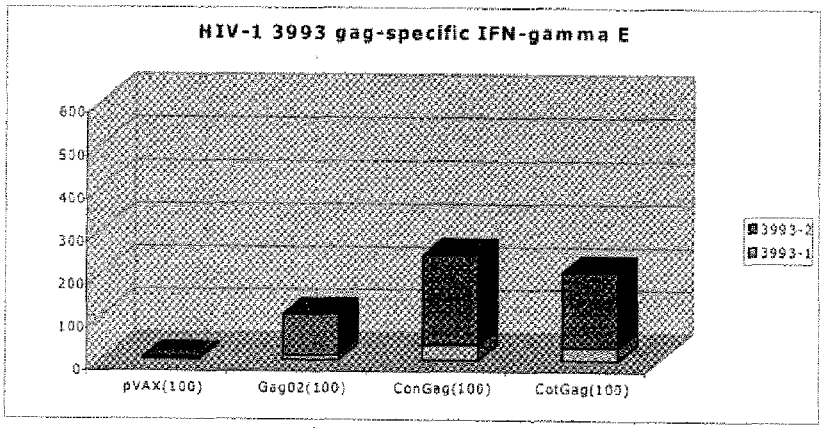
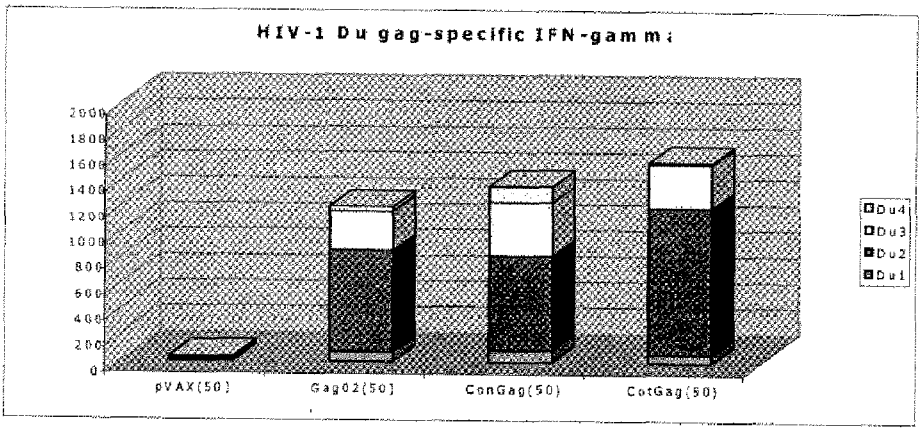


Figure 20
Panel D



Panel E



Panel F

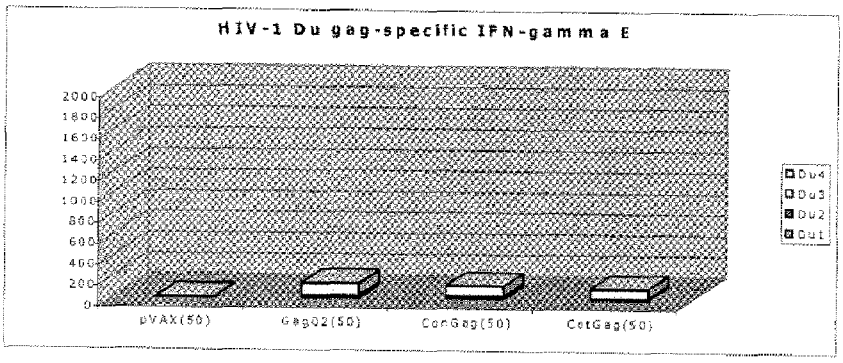
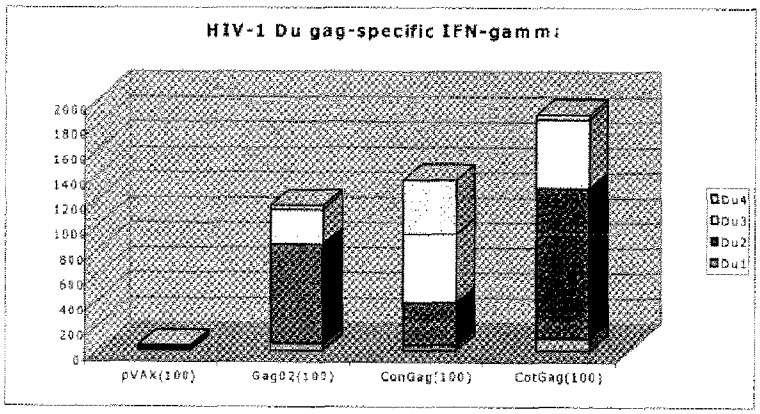
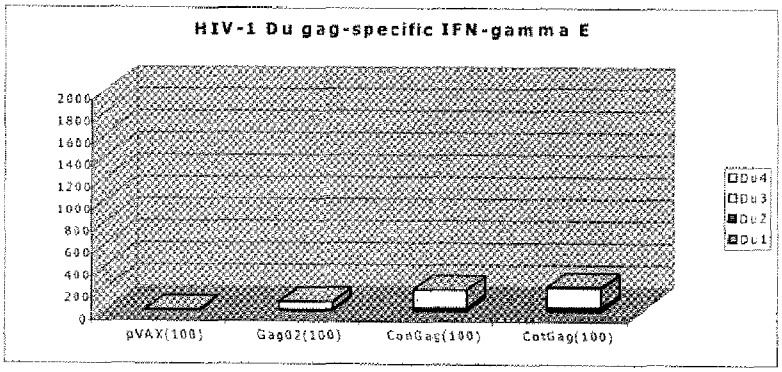


Figure 20
Panel G



Panel H



Panel I

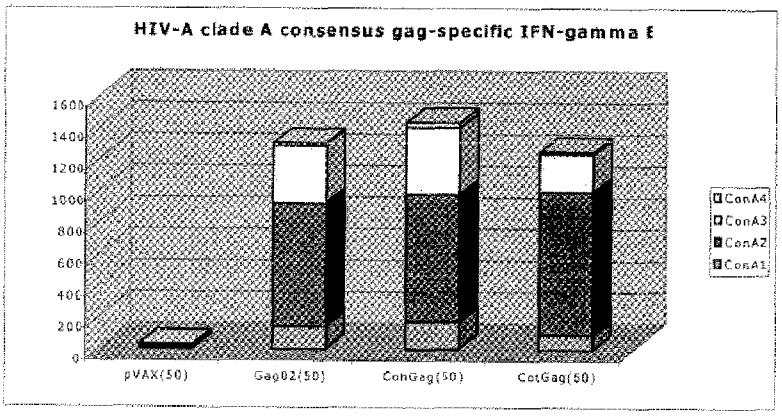
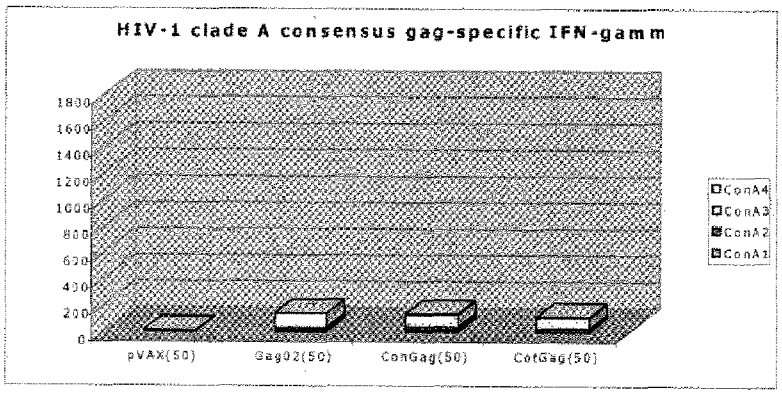
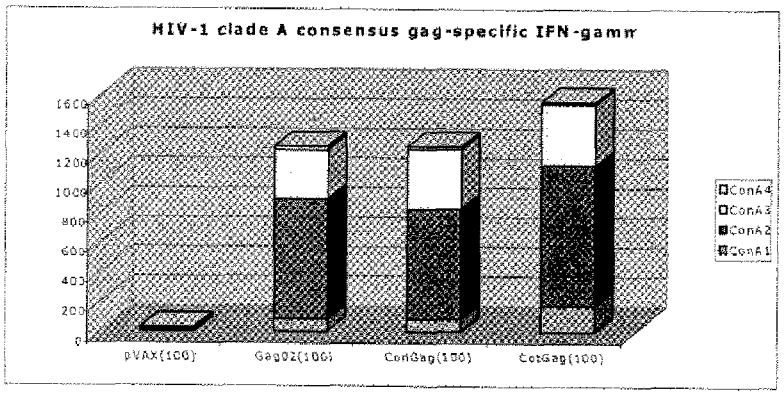


Figure 20
Panel J



Panel K



Panel L

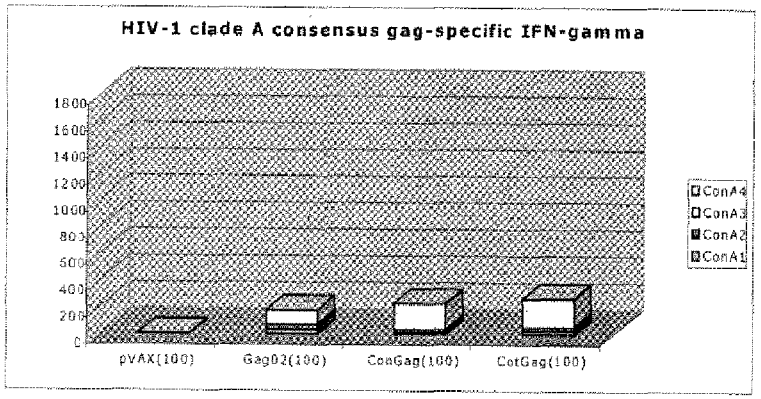
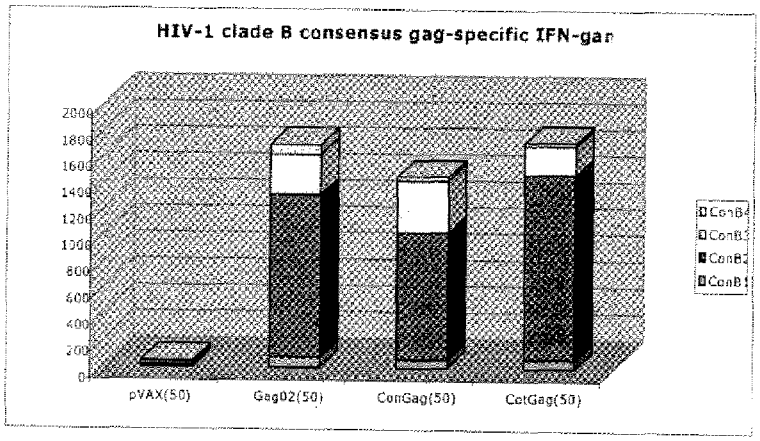
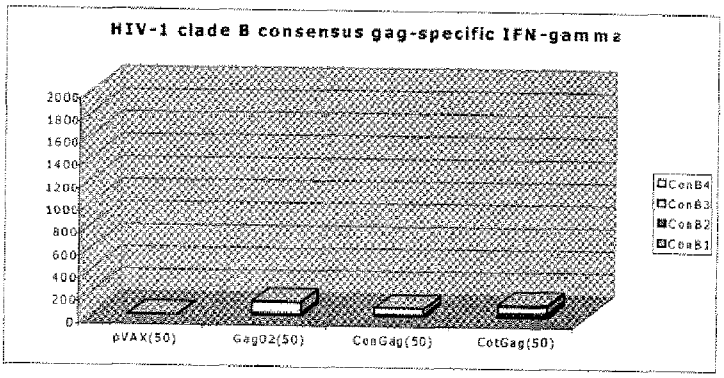


Figure 20
Panel M



Panel N



Panel O

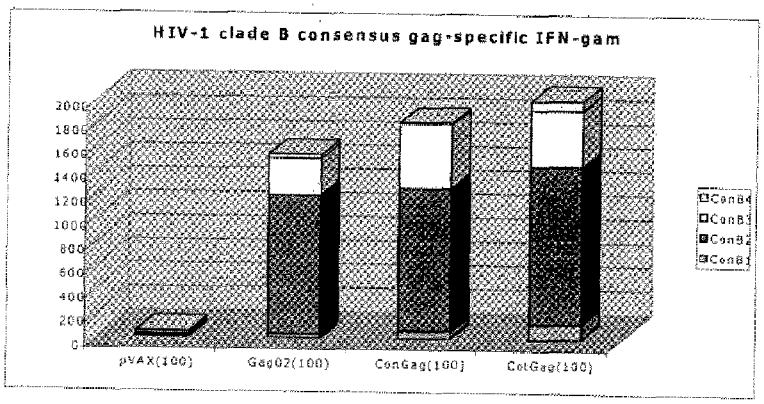
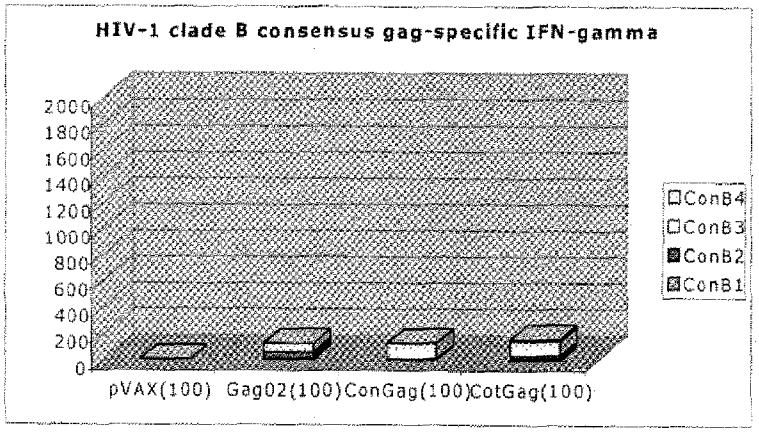
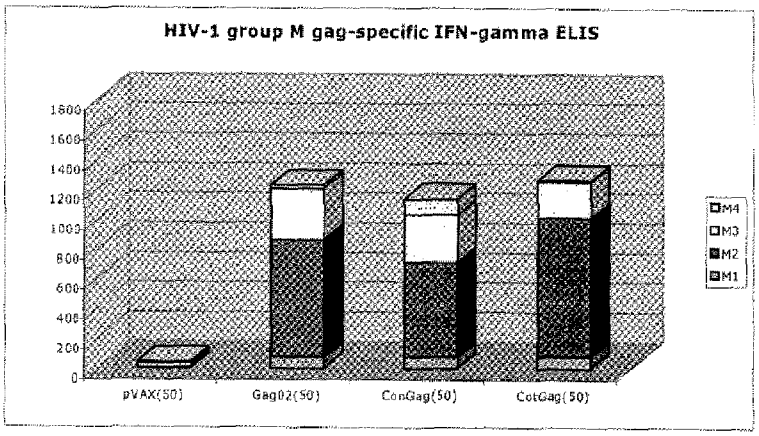


Figure 20
Panel P



Panel Q



Panel R

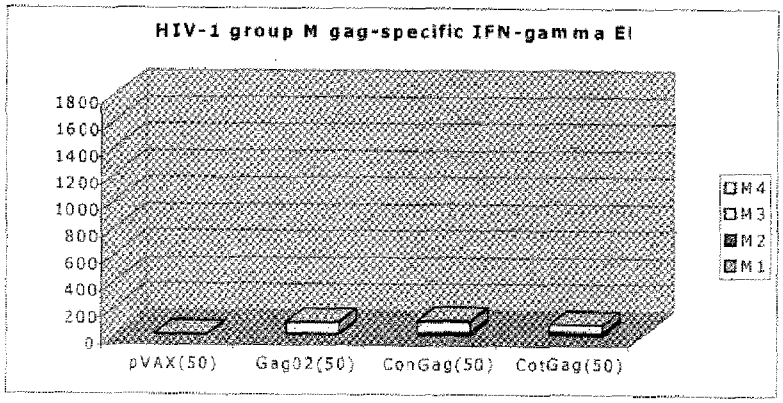
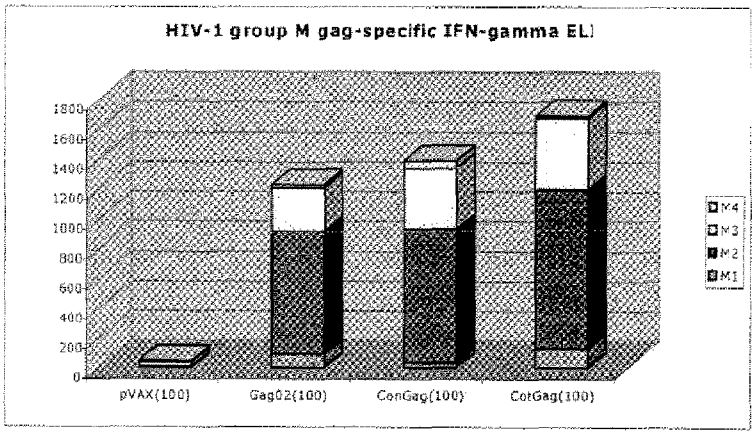
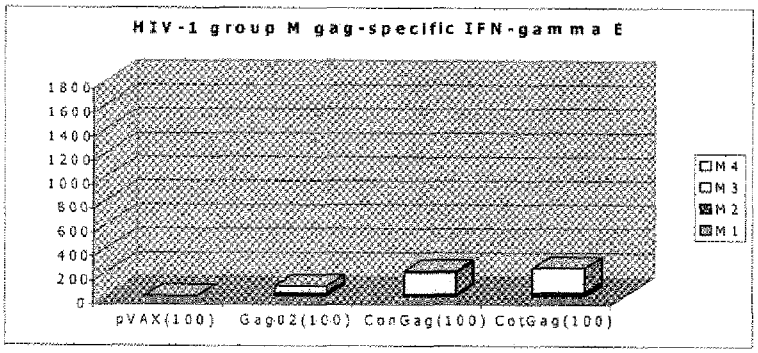


Figure 20
Panel S



Panel T



Panel U

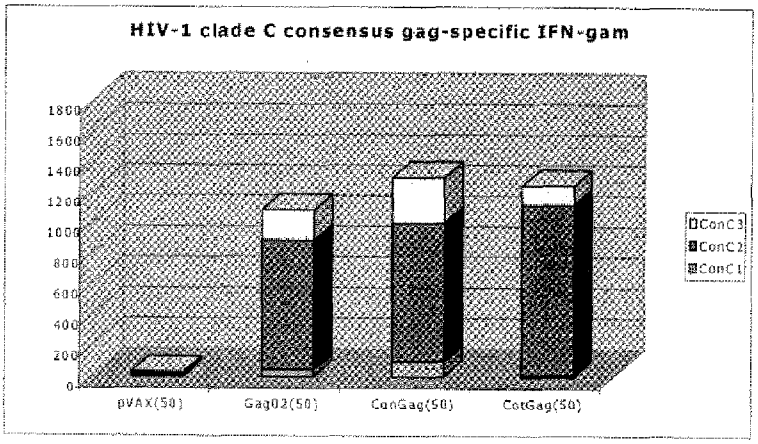
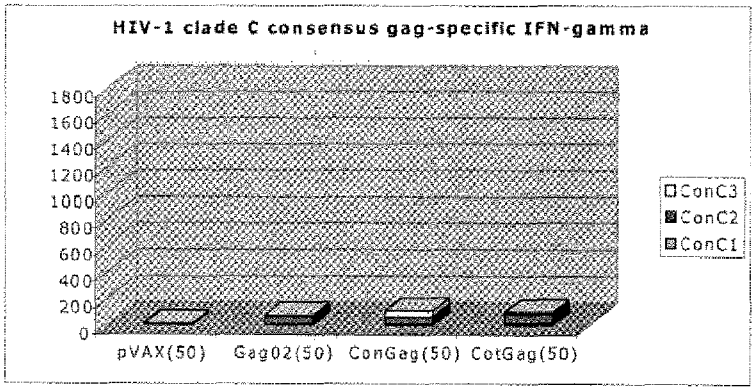
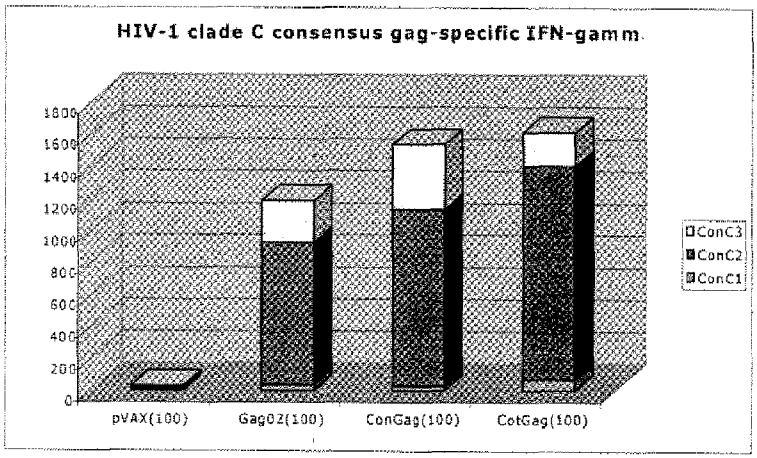


Figure 20
Panel V



Panel W



Panel X

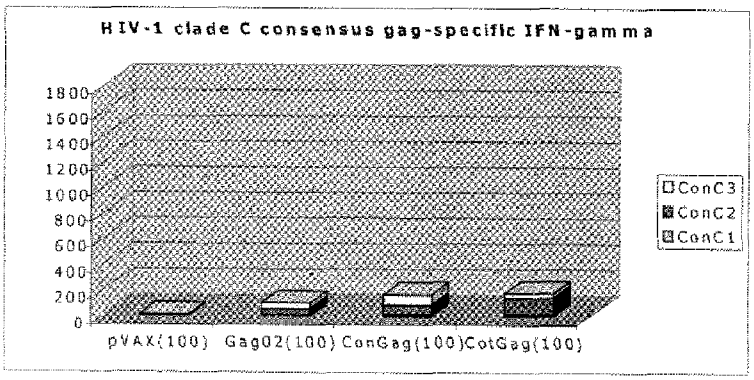


Figure 21

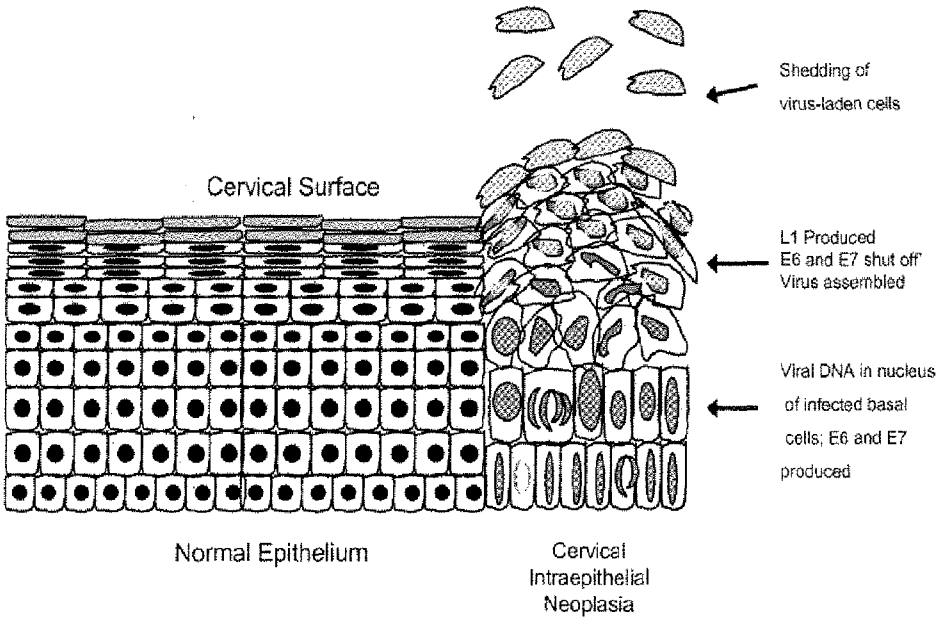


Figure 22

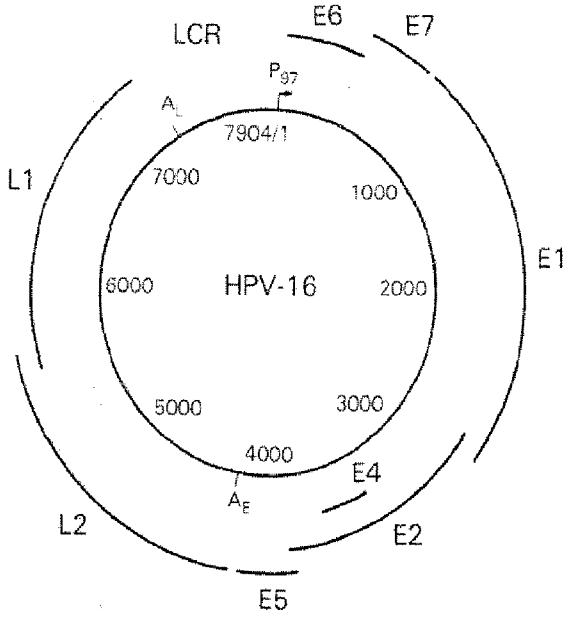


Figure 23

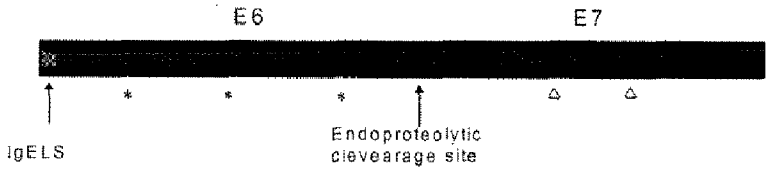
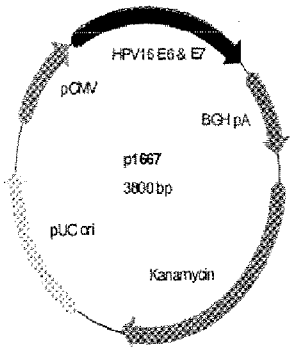


Figure 24



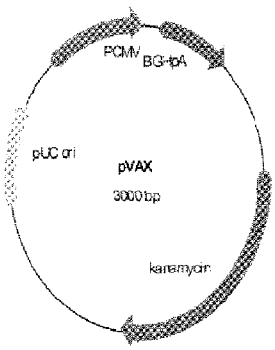
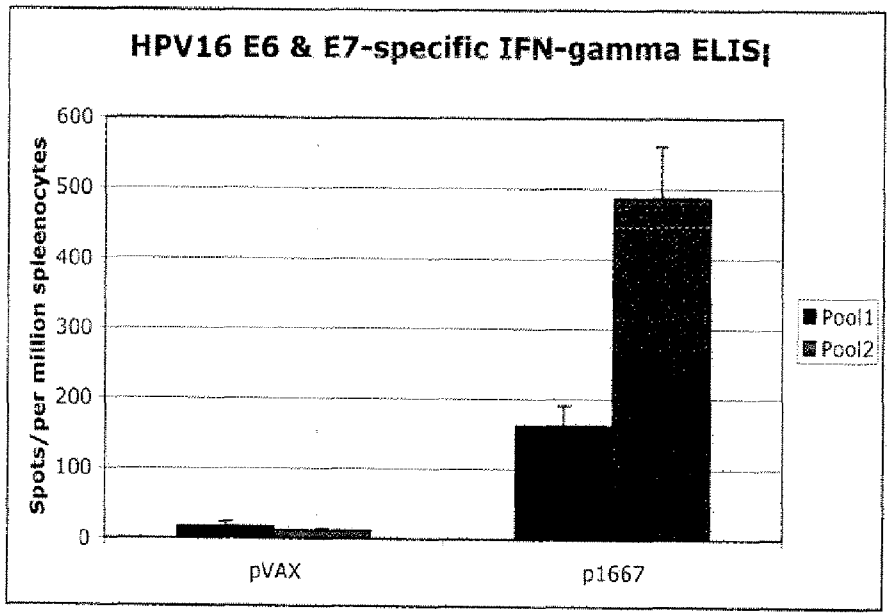


Figure 25
Panel A



Panel B

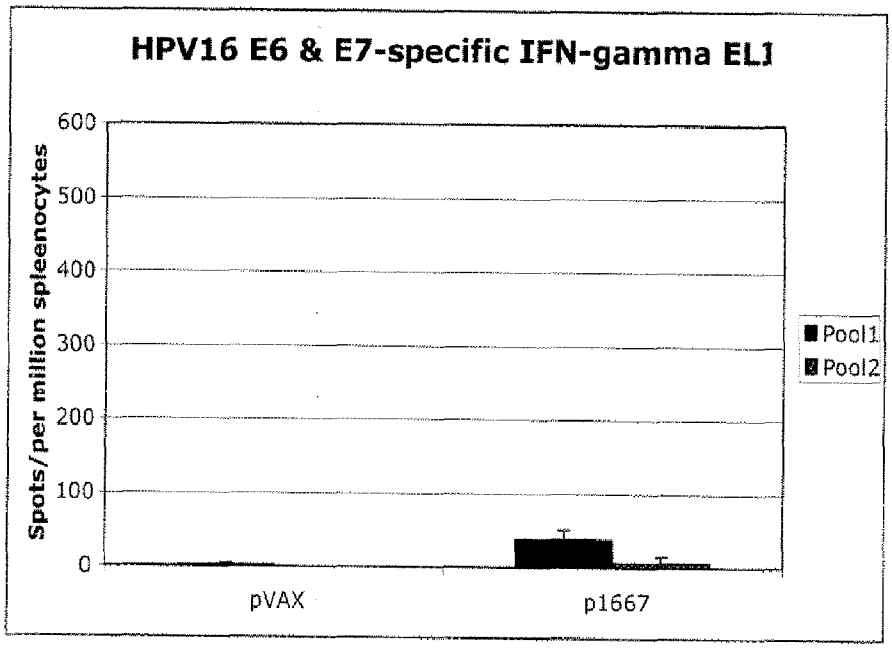
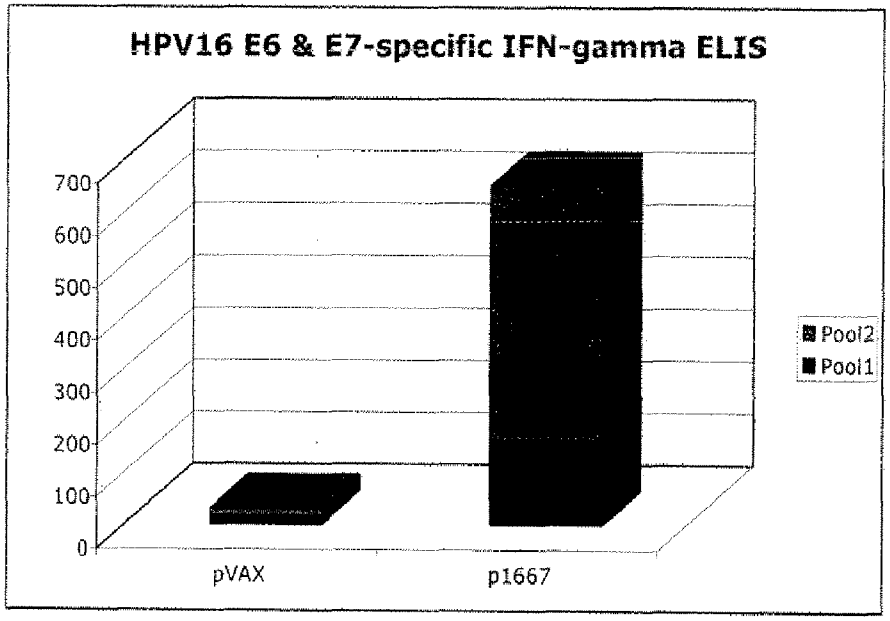


Figure 25
Panel C



Panel D

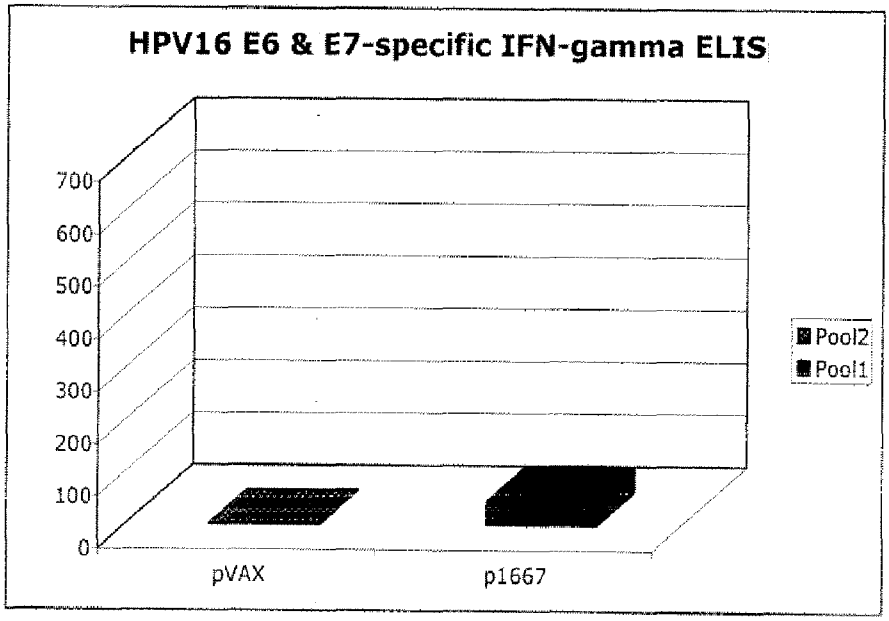
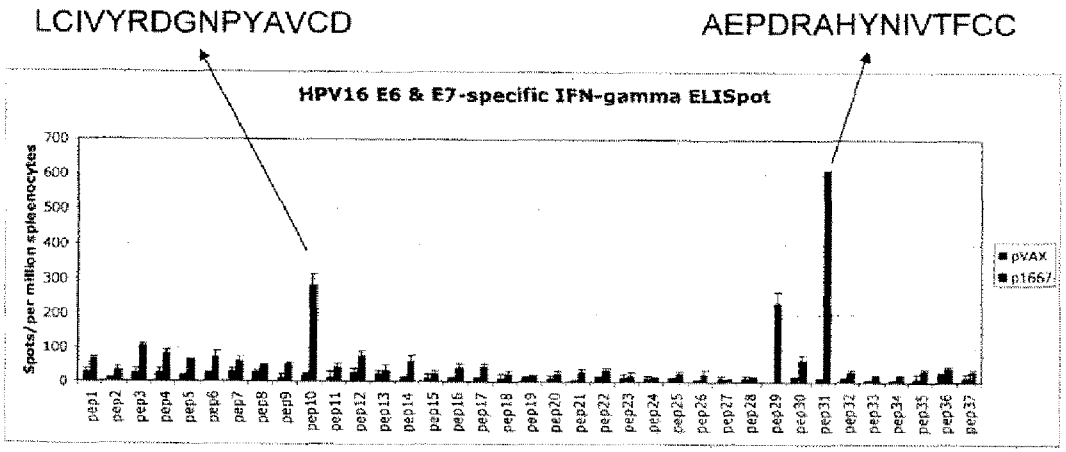


Figure 26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Spots/ per million splenocytes
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600
500
400
300
200
100
0
pep1 pep2 pep3 pep4 pep5 pep6 pep7 pep8 pep9 pep10 pep11 pep12 pep13 pep14 pep15 pep16 pep17 pep18 pep19 pep20 pep21 pep22 pep23 pep24 pep25 pep26 pep27 pep28 pep29 pep30 pep31 pep32 pep33 pep34 pep35 pep36 pep37
pVAX
p166/

Figure 27

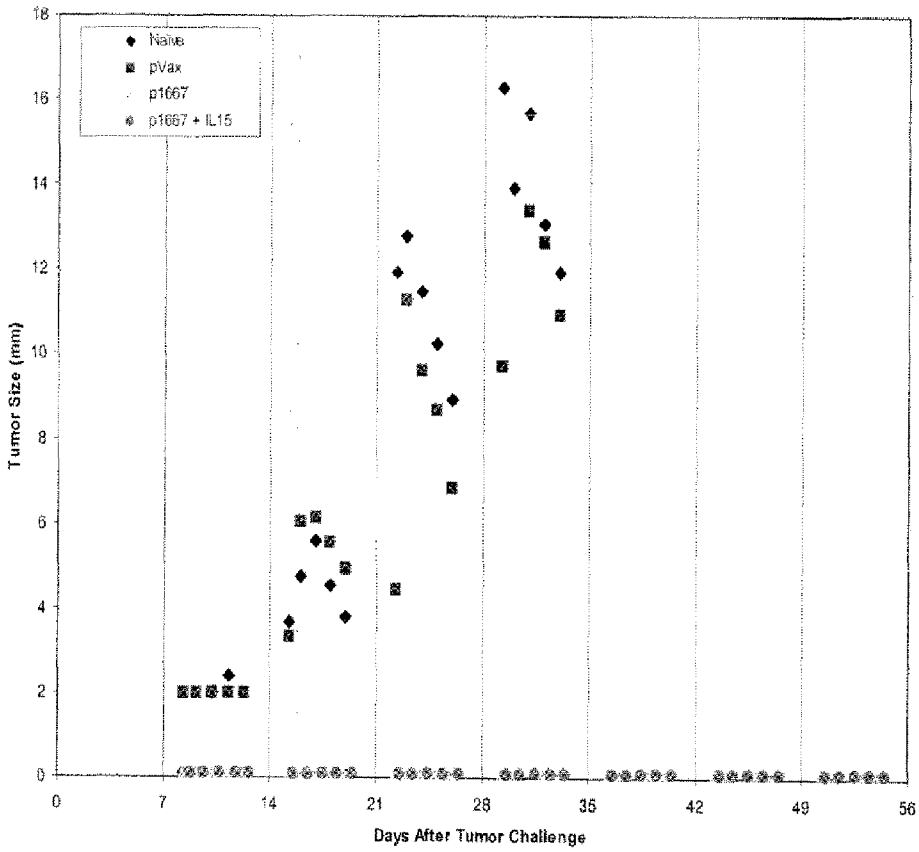


Figure 28

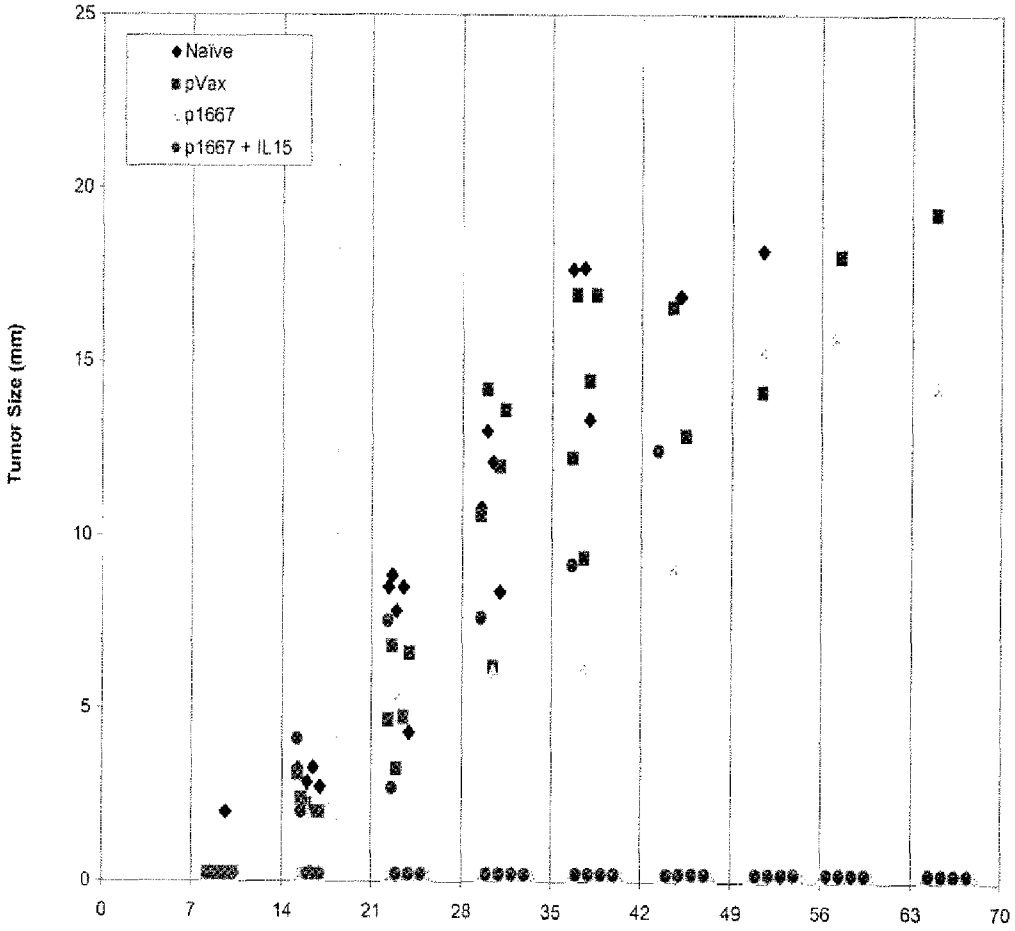


Figure 29

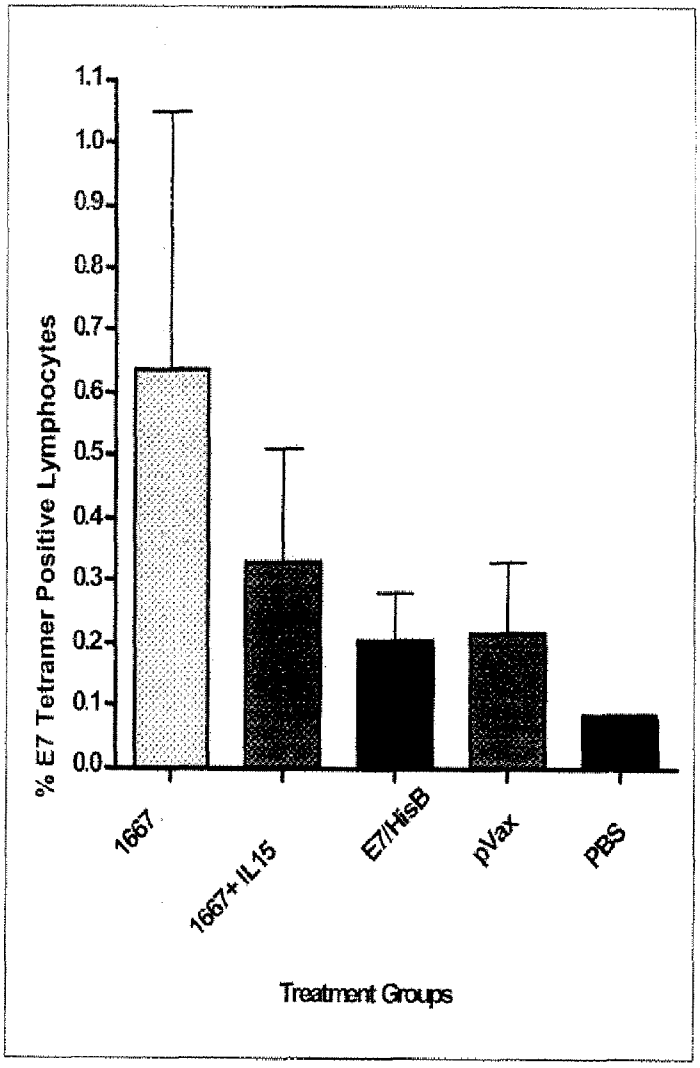


Figure 30

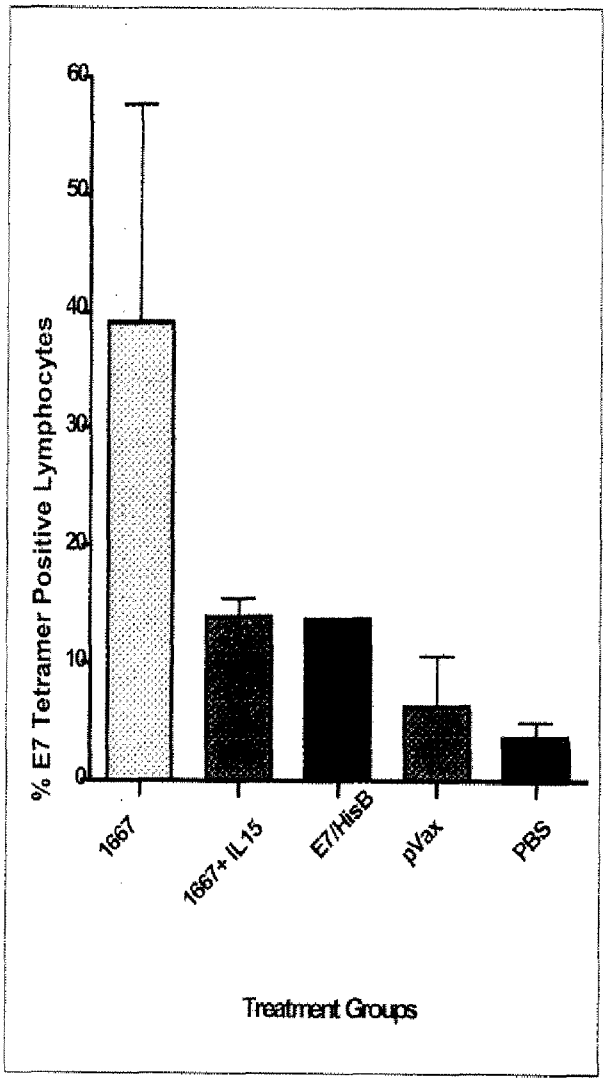


Figure 31

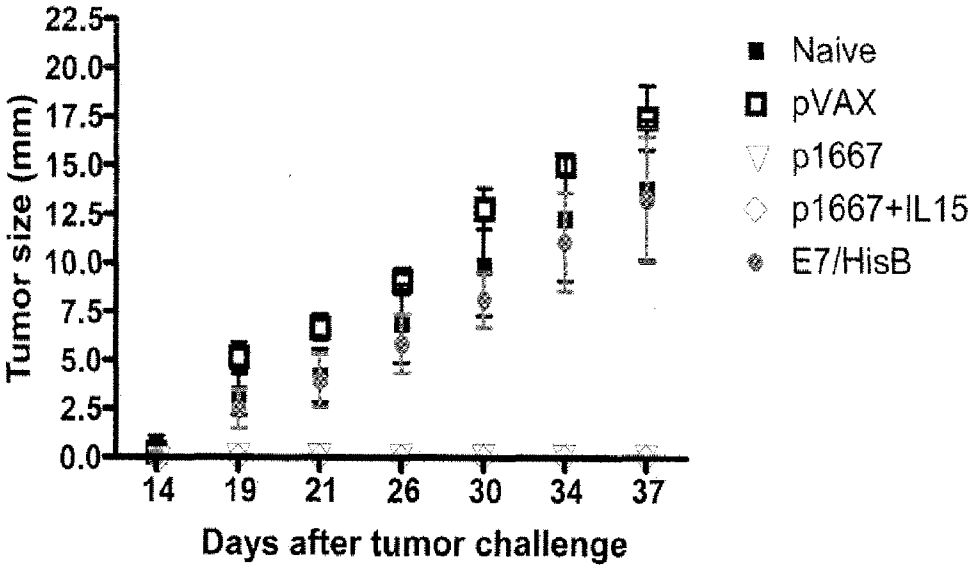


Figure 32

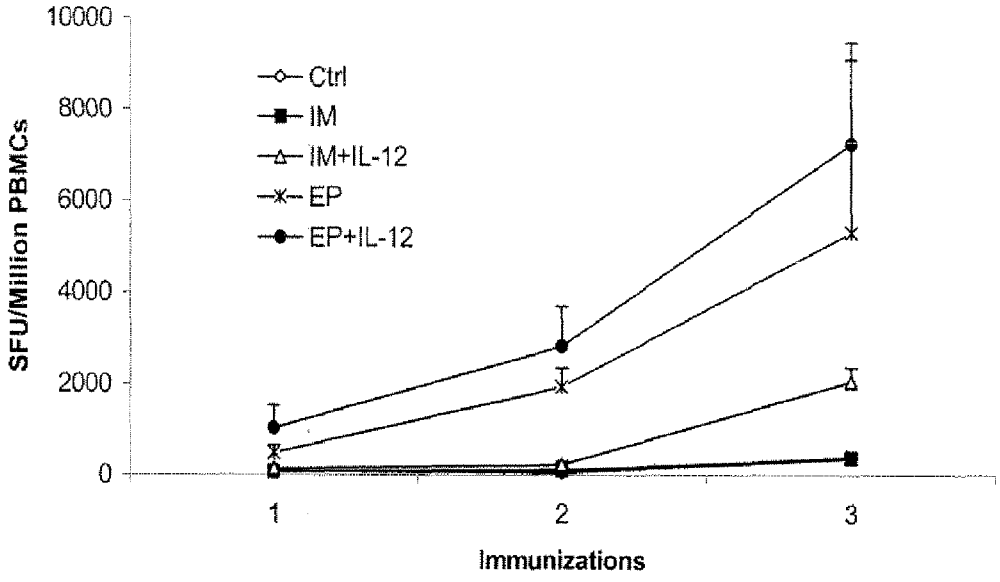


Figure 33

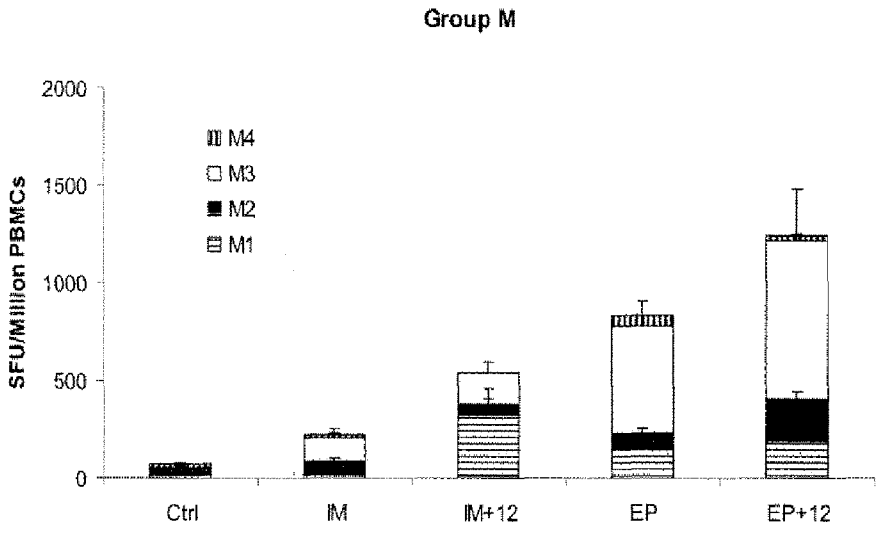
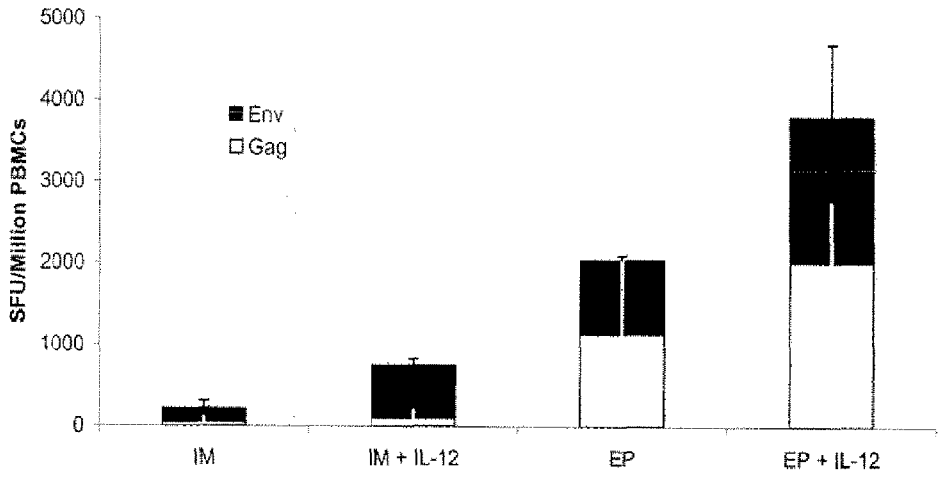


Figure 34



Sequence Listing

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1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2024-02-26
1-6	Original free text language code	en
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	AU
2-2	Current application: Application number	2024200014
2-3	Current application: Filing date	2024-01-02
2-4	Current application: Applicant file reference	206108-0003-07AU
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	US 60/833861
2-7	Earliest priority application: Filing date	2006-07-28
2-8en	Applicant name	The Trustees of the University of Pennsylvania
2-8	Applicant name: Name Latin	
2-9en	Inventor name	David Weiner
2-9	Inventor name: Name Latin	
2-10en	Invention title	IMPROVED VACCINES AND METHODS FOR USING THE SAME
2-11	Sequence Total Quantity	43

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3-3	**Sequences**	
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3-3-2	Molecule Type	DNA
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		organism=synthetic construct
	NonEnglishQualifier Value	
3-3-5	Residues	ggatccgcca ccatggactg gacctggatt ctgttcctgg tggccgccgc caccagagtg 60 cacagcagag tgaagggcat ccggaagaac taccagcacc tgtggagatg gggcaccatg 120 ctgctgggca tgctgatgat ctgttctgcc gccgagaagc tgtgggtgac cgtgtactac 180 ggcgtgcctg tgtggaagga ggccaccacc accctgttct gcgccagcga cgccaaggcc 240 tacgataccg aagtgcacaa tgtgtgggcc acccacgcct gcgtgcctac cgatcccaac 300 cctcaggaag tggtgctgga gaacgtgacc gagaacttca acatgtggaa gaacaacatg 360 gtggagcaga tgcacgagga catcatcagc ctgtgggacc agagcctgaa gccttgcgtg 420 aagctgaccc ctctgtgcgt gaccctgaac tgcaccgacc tgagcggcga gaagatggag 480 aagggcgaga tcaagaactg cagcttcaac atcaccacct ccatccggga caaagtgcag 540 aaggagtacg ccctgttcta caagctggac gtggtgccca tcgacaacga caacaccagc 600 taccggctga tcagctgcaa caccagcgtg atcacccagg cctgccccaa agtgagcttc 660 gagcccatcc ccatccacta ctgcgccccc gccggcttcg ccatcctgaa gtgcaacgac 720 aagaagttca acggcaccgg cccttgcacc aatgtgagca ccgtgcagtg cacccacggc 780 atcagacccg tggtgtccac ccagctgctg ctgaacggca gcctggccga ggaagaagtg 840 gtgatccgga gcgagaattt caccaacaac gccaagacca tcatcgtgca gctgaacgag 900 agcgtggaga tcaactgcac ccggcccaac aacaataccc ggaagagcat ccacatcggc 960 cctggccagg ccttctacac caccggcgag atcatcggcg atatcaggca ggcccactgc 1020 aatatcagcc gggccaagtg gaacaacacc ctgaagcaga tcgtgaagaa gctgcgggag 1080 cagttcggca acaagaccat cgtgttcaac cagagcagcg gcggcagacc tagaatcgtg 1140 atgcacagct tcaactgtgg cggcgagttc ttctactgca acacaaccca gctgttcaac 1200 agcacctgga acgtgaacgg gacctggaac aacaacaccg agggcaacga caccatcacc 1260 ctgccctgcc ggatcaagca gatcatcaat atgtggcagg aggtgggcaa ggccatgtac 1320 gcccctccca tcagaggcca gatccggtgc agcagcaata tcaccggcct gctgctgacc 1380 agagatggcg gcaacaataa caccaacgag accgagatct ttagacctgg cggcggagac 1440 atgagggaca actggcggag cgagctgtac aagtacaaag tggtgaagat cgagcccctg 1500 ggcgtggccc ccaccaaggc caagagaaga gtggtgcagc gggagaagag agctgtgggc 1560 atcggcgcca tgtttctggg ctttctggga gccgccggaa gcaccatggg agccgccagc 1620 atgaccctga ccgtgcaggc cagacagctg ctgagcggca tcgtgcagca gcagaacaac 1680 ctgctgagag ccatcgaggc ccagcagcac ctgctgcagc tgacagtgtg gggcatcaag 1740 cagctgcagg cccgcgtgct ggccgtggag agatacctga aggaccagca gctgctggga 1800 atctggggct gcagcggcaa gctgatctgc accaccaccg tgccctggaa cgccagctgg 1860 agcaacaaga gcctggacga gatctgggac aacatgacct ggatggagtg ggagcgggag 1920 atcgacaact acaccagcct gatctacacc ctgatcgagg agagccagaa ccagcaggag 1980 aagaacgagc aggagctgct ggagctggac aagtgggcca gcctgtggaa ctggttcgac 2040 atcaccaact ggctgtggta catcaagatc ttcatcatga ttgtgggcgg cctgatcggc 2100 ctgagaatcg tgttcgccgt gctgagcatc tacccctacg acgtgcccga ttacgcctga 2160 gaattcgtaa gtaagtgtca tatgggagag ctcgactaga ctggacagcc aatgacgggt 2220 aagagagtga catttctcac taacctaaga caggagggcc gtcaaagcta ctgcctaatc 2280 caatgacggg taatagtgac aagaaatgta tcactccaac ctaagacagg cgcagcctcc 2340 gagggatgtg tcttttgttt tttataatta aaaagggtga catgtccgga gccgtgctgc 2400 ccggatgatg tcttggcctc tgtttgctac cggtatcgat gttaacgtcg accccgggct 2460 cgaggtaagt aagtgtcata tgggagagct cgactagact ggacagccaa tgacgggtaa 2520 gagagtgaca tttctcacta acctaagaca ggagggccgt caaagctact gcctaatcca 2580 atgacgggta atagtgacaa gaaatgtatc actccaacct aagacaggcg cagcctccga 2640 gggatgtgtc ttttgttttt tataattaaa aagggtgaca tgtccggagc cgtgctgccc 2700 ggatgatgtc ttggcctctg tttgctgcgg ccgc 2734
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	AA
3-4-3	Length	715
3-4-4	Features	**REGION 1..715**
	Location/Qualifiers	note=Subtype B consensus Envelope protein sequence construct
		source 1..715
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-4-5	Residues	MDWTWILFLV AAATRVHSRV KGIRKNYQHL WRWGTMLLGM LMICSAAEKL WVTVYYGVPV 60 WKEATTTLFC ASDAKAYDTE VHNVWATHAC VPTDPNPQEV VLENVTENFN MWKNNMVEQM 120 HEDIISLWDQ SLKPCVKLTP LCVTLNCTDL SGEKMEKGEI KNCSFNITTS IRDKVQKEYA 180 LFYKLDVVPI DNDNTSYRLI SCNTSVITQA CPKVSFEPIP IHYCAPAGFA ILKCNDKKFN 240 GTGPCTNVST VQCTHGIRPV VSTQLLLNGS LAEEEVVIRS ENFTNNAKTI IVQLNESVEI 300 NCTRPNNNTR KSIHIGPGQA FYTTGEIIGD IRQAHCNISR AKWNNTLKQI VKKLREQFGN 360 KTIVFNQSSG GRPRIVMHSF NCGGEFFYCN TTQLFNSTWN VNGTWNNNTE GNDTITLPCR 420 IKQIINMWQE VGKAMYAPPI RGQIRCSSNI TGLLLTRDGG NNNTNETEIF RPGGGDMRDN 480 WRSELYKYKV VKIEPLGVAP TKAKRRVVQR EKRAVGIGAM FLGFLGAAGS TMGAASMTLT 540 VQARQLLSGI VQQQNNLLRA IEAQQHLLQL TVWGIKQLQA RVLAVERYLK DQQLLGIWGC 600 SGKLICTTTV PWNASWSNKS LDEIWDNMTW MEWEREIDNY TSLIYTLIEE SQNQQEKNEQ 660 ELLELDKWAS LWNWFDITNW LWYIKIFIMI VGGLIGLRIV FAVLSIYPYD VPDYA 715
3-5	**Sequences**	

3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	DNA
3-5-3	Length	2140
3-5-4	Features	**misc_feature 1..2140**
	Location/Qualifiers	note=Subtype C consensus Envelope DNA sequence construct
		source 1..2140
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-5-5	Residues	ggatccgcca ccatggattg gacctggatt ctgttcctgg tggccgccgc cacaagagtg 60 cacagcagag tgcggggcat cctgagaaat tgccagcagt ggtggatctg gggcattctg 120 gggttctgga tgctgatgat ctgcaacgtg atgggcaacc tgtgggtgac cgtgtactac 180 ggcgtgcctg tgtggaagga ggccaagacc accctgttct gtgccagcga tgccaaggcc 240 tacgagaccg aggtgcacaa tgtgtgggcc acccacgcct gtgtgcccac cgatcccaac 300 cctcaggaga tggtgctgga gaacgtgacc gagaacttca acatgtggaa gaacgacatg 360 gtggaccaga tgcacgagga catcatcagc ctgtgggacc agagcctgaa gccttgcgtg 420 aagctgaccc ctctgtgcgt gaccctgaac tgccggaaca acgtgaacaa caacaacacc 480 atgaaggagg agatcaagaa ctgcagcttc aacatcacca ccgagctgcg ggacaagaag 540 cagaaggtgt acgccctgtt ctaccggctg gacatcgtgc ccctgaacga gaagaacaac 600 agcaacgact accggctgat caactgcaac accagcgcca tcacccaggc ctgtcccaag 660 gtgtccttcg accccatccc catccactat tgtgcccctg ccggctacgc catcctgaag 720 tgcaacaaca agaccttcaa cggcaccggc ccctgcaata atgtgagcac cgtgcagtgt 780 acccacggca tcaagcctgt ggtgtccacc cagctgctgc tgaatggcag cctggccgag 840 gaggagatta tcatccggag cgagaacctg accaacaacg ccaagaccat cattgtgcac 900 ctgaatgaga gcgtggagat cgtgtgtacc cggcccaaca acaatacccg gaagagcatc 960 agaatcggcc ctggccagac cttttacgcc accggcgaca tcatcggcga tatcaggcag 1020 gcccactgca atatcagcga ggagaagtgg aacaagaccc tgcagcgggt gtccgagaag 1080 ctgaaggagc acttccccaa taagaccatc aagttcgccc ctagcagcgg cggcagactg 1140 gagatcacca cccacagctt caactgcagg ggcgagttct tctactgcaa taccagcaag 1200 ctgttcaaca gcacctacat gcccaacagc accaacaata ccaacaccac catcaccctg 1260 ccctgccgga tcaagcagat catcaatatg tggcaggaag tgggcagagc catgtacgcc 1320 cctcccatcg agggcaacat cacctgcaag tccaacatca ccggcctgct gctgacaaga 1380 gatggcggca agaacgacac caatgacacc gagaccttca gacctggcgg cggagacatg 1440 agggacaact ggcggagcga gctgtacaag tacaaggtgg tggagatcaa gcctctgggc 1500 gtggccccta ccaaggccaa gaggagagtg gtggagaggg agaagagagc cgtgggcatc 1560 ggcgccgtgt ttctgggctt tctgggagcc gccggatcta caatgggagc cgccagcatc 1620 acactgaccg tgcaggccag acagctgctg agcggcatcg tgcagcagca gagcaatctg 1680 ctgagagcca tcgaggccca gcagcacatg ctgcagctga cagtgtgggg catcaagcag 1740 ctgcagacca gagtgctggc catcgagcgc tacctgaagg atcagcagct gctgggcatc 1800 tggggctgta gcggcaagct gatctgtacc accgccgtgc cttggaatag cagctggagc 1860 aacaagagcc aggaggacat ctgggacaac atgacctgga tgcagtggga ccgggagatc 1920 agcaactaca ccgacaccat ctacaggctg ctggaggaca gccagaacca gcaggagaag 1980 aacgagaagg acctgctggc cctggacagc tggaagaacc tgtggaactg gttcgacatc 2040 accaactggc tgtggtacat caagatcttc atcatgattg tgggcggcct gatcggcctg 2100 agaatcatct tcgccgtgct gagcatctga tagcggccgc 2140
3-6	**Sequences**	
3-6-1	Sequence Number [ID]	6
3-6-2	Molecule Type	AA
3-6-3	Length	705
3-6-4	Features	**REGION 1..705**
	Location/Qualifiers	note=Subtype C consensus Envelope protein sequence construct
		source 1..705
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-6-5	Residues	MDWTWILFLV AAATRVHSRV RGILRNCQQW WIWGILGFWM LMICNVMGNL WVTVYYGVPV 60 WKEAKTTLFC ASDAKAYETE VHNVWATHAC VPTDPNPQEM VLENVTENFN MWKNDMVDQM 120 HEDIISLWDQ SLKPCVKLTP LCVTLNCRNN VNNNNTMKEE IKNCSFNITT ELRDKKQKVY 180 ALFYRLDIVP LNEKNNSNDY RLINCNTSAI TQACPKVSFD PIPIHYCAPA GYAILKCNNK 240 TFNGTGPCNN VSTVQCTHGI KPVVSTQLLL NGSLAEEEII IRSENLTNNA KTIIVHLNES 300 VEIVCTRPNN NTRKSIRIGP GQTFYATGDI IGDIRQAHCN ISEEKWNKTL QRVSEKLKEH 360 FPNKTIKFAP SSGGRLEITT HSFNCRGEFF YCNTSKLFNS TYMPNSTNNT NTTITLPCRI 420 KQIINMWQEV GRAMYAPPIE GNITCKSNIT GLLLTRDGGK NDTNDTETFR PGGGDMRDNW 480 RSELYKYKVV EIKPLGVAPT KAKRRVVERE KRAVGIGAVF LGFLGAAGST MGAASITLTV 540 QARQLLSGIV QQQSNLLRAI EAQQHMLQLT VWGIKQLQTR VLAIERYLKD QQLLGIWGCS 600 GKLICTTAVP WNSSWSNKSQ EDIWDNMTWM QWDREISNYT DTIYRLLEDS QNQQEKNEKD 660 LLALDSWKNL WNWFDITNWL WYIKIFIMIV GGLIGLRIIF AVLSI 705
3-7	**Sequences**	
3-7-1	Sequence Number [ID]	7
3-7-2	Molecule Type	DNA
3-7-3	Length	2089
3-7-4	Features	**misc_feature 1..2089**
	Location/Qualifiers	note=Subtype D consensus Envelope DNA sequence construct

		source 1..2089
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-7-5	Residues	gggcatcaag cggaattacc agcacctgtg gaagtggggc accatgctgc tgggcatgct 60 gatgacctgc agcgtggccg agaacctgtg ggtgaccgtg tactacggcg tgcctgtgtg 120 gaaggaagcc accaccaccc tgttctgcgc cagcgatgcc aagagctaca agaccgaggc 180 ccacaatatc tgggccaccc acgcctgcgt gcctaccgat cccaaccctc aggagatcga 240 gctggagaac gtgaccgaga acttcaacat gtggaagaac aacatggtgg agcagatgca 300 cgaggacatc atcagcctgt gggaccagag cctgaagcct tgcgtgaagc tgacccctct 360 gtgcgtgacc ctgaactgca ccgacggcat gaggaacgac accaacgata ccaacgtgac 420 catggaggag ggcgagatga agaactgcag cttcaacatc accaccgaag tgcgggacaa 480 gaagaagcag gtgcacgccc tgttctacaa gctggacgtg gtgcccatcg acgacaacaa 540 caccaacaac agcaactacc ggctgatcaa ctgcaacacc agcgccatca cccaggcctg 600 ccccaaagtg accttcgagc ccatccccat ccactactgc gcccctgccg gcttcgccat 660 cctgaagtgc aaggataaga agttcaacgg caccggcccc tgcaagaatg tgagcaccgt 720 gcagtgcacc cacggcatca gacccgtggt gtccacccag ctgctgctga acggcagcct 780 ggccgaggag gagatcatca tccggagcga gaacctgacc aacaacgcca agatcatcat 840 tgtgcagctg aacgagagcg tgaccatcaa ttgcacccgg ccctacaaca atacccggaa 900 gcgcatcccc atcggcctgg gccaggcctt ctacaccacc agaggcatca tcggcgacat 960 cagacaggcc cactgcaata tcagcggagc cgagtggaat aagaccctgc agcaggtggc 1020 caagaagctg ggcgacctgc tgaacaagac caccatcatc ttcaagccta gcagcggcgg 1080 cagacctaga atcaccaccc acagcttcaa ttgtggcggc gagttcttct actgcaatac 1140 cagccggctg ttcaacagca cctggagcaa gaacagcacc agcaactcca ccaaggagaa 1200 caacaccatc accctgccct gccggatcaa gcagatcatc aatatgtggc agggagtggg 1260 caaggccatg tacgcccctc ccatcgaggg cctgatcaag tgcagcagca acatcaccgg 1320 cctgctgctg accagagatg gcggagccaa caactcccac aacgagacct tcagacctgg 1380 cggcggagac atgagggaca actggcggag cgagctgtac aagtacaaag tggtgaagat 1440 cgagcccctg ggcgtggccc ccaccagagc caagagaaga gtggtggagc gggagaagag 1500 agccatcgga ctgggcgcca tgttcctggg cttcctggga gccgccggaa gcaccatggg 1560 agccgccagc ctgaccctga ccgtgcaggc cagacagctg ctgagcggca tcgtgcagca 1620 gcagaacaac ctgctgagag ccattgaggc ccagcagcac ctgctgcagc tgacagtgtg 1680 gggcattaag cagctgcagg ccaggattct ggccgtggag cgctacctga aggatcagca 1740 gctgctggga atctggggct gcagcggcaa gcacatctgc accaccaccg tgccttggaa 1800 tagcagctgg agcaacaaga gcctggacga gatctggaac aacatgacct ggatggagtg 1860 ggagagggag atcgacaact acaccggcct gatctacagc ctgatcgagg agagccagac 1920 ccagcaggag aagaacgagc aggagctgct ggagctggac aagtgggcca gcctgtggaa 1980 ctggttcagc atcacccagt ggctgtggta catcaagatc ttcatcatga ttgtgggcgg 2040 cctgatcggc ctgagaatcg tgttcgccgt gctgagcctg tgactcgag 2089
3-8	**Sequences**	
3-8-1	Sequence Number [ID]	8
3-8-2	Molecule Type	AA
3-8-3	Length	714
3-8-4	Features	**REGION 1..714**
	Location/Qualifiers	note=Subtype D consensus Envelope protein sequence construct
		source 1..714
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-8-5	Residues	MDWTWILFLV AAATRVHSRV RGIKRNYQHL WKWGTMLLGM LMTCSVAENL WVTVYYGVPV 60 WKEATTTLFC ASDAKSYKTE AHNIWATHAC VPTDPNPQEI ELENVTENFN MWKNNMVEQM 120 HEDIISLWDQ SLKPCVKLTP LCVTLNCTDG MRNDTNDTNV TMEEGEMKNC SFNITTEVRD 180 KKKQVHALFY KLDVVPIDDN NTNNSNYRLI NCNTSAITQA CPKVTFEPIP IHYCAPAGFA 240 ILKCKDKKFN GTGPCKNVST VQCTHGIRPV VSTQLLLNGS LAEEEIIIRS ENLTNNAKII 300 IVQLNESVTI NCTRPYNNTR KRIPIGLGQA FYTTRGIIGD IRQAHCNISG AEWNKTLQQV 360 AKKLGDLLNK TTIIFKPSSG GRPRITTHSF NCGGEFFYCN TSRLFNSTWS KNSTSNSTKE 420 NNTITLPCRI KQIINMWQGV GKAMYAPPIE GLIKCSSNIT GLLLTRDGGA NNSHNETFRP 480 GGGDMRDNWR SELYKYKVVK IEPLGVAPTR AKRRVVEREK RAIGLGAMFL GFLGAAGSTM 540 GAASLTLTVQ ARQLLSGIVQ QQNNLLRAIE AQQHLLQLTV WGIKQLQARI LAVERYLKDQ 600 QLLGIWGCSG KHICTTTVPW NSSWSNKSLD EIWNNMTWME WEREIDNYTG LIYSLIEESQ 660 TQQEKNEQEL LELDKWASLW NWFSITQWLW YIKIFIMIVG GLIGLRIVFA VLSL 714
3-9	**Sequences**	
3-9-1	Sequence Number [ID]	9
3-9-2	Molecule Type	DNA
3-9-3	Length	1049
3-9-4	Features	**misc_feature 1..1049**
	Location/Qualifiers	note=Subtype B consensus Nef-Rev DNA sequence construct
		source 1..1049
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-9-5	Residues	ggatccgcca ccatggactg gacctggatt ctgttcctgg tggccgctgc caccagagtg 60 cacagcagca agagaagcgt ggtgggttgg cctacagtgc gggagaggat gagaagagcc 120

		gagcctgccg ccgatggagt gggcgccgtg tctagagatc tggagaagca cggcgccatc 180 accagcagca ataccgccgc caacaatgcc gactgcgcct ggctggaggc ccaggaggag 240 gaggaagtgg gcttccctgt gagagcccag gtggccctga gagccatgac ctacaaggcc 300 gccgtggatc tgagccactt cctgaaggag aagggcggcc tggagggcct gatctacagc 360 cagaagcggc aggacatcct ggatctgtgg gtgtaccaca cccagggcta cttccccgac 420 tggcagaatt acacccctgg ccctggcatc agataccctc tgaccttcgg ctggtgcttc 480 aagctggtgc ctgtggagcc tgagaaagtg gaggaggcca acgagggcga gaacaattct 540 gccgcccacc ctatgagcct gcacggcatg gacgatcccg agagggaagt gctggtgtgg 600 aagttcgaca gcaggctggc cttccaccac atggccagag agctgcaccc cgagtactac 660 aaggactgcc ggggcaggaa gagaagaagc gccggcagaa gcggcgacag cgacgaggag 720 ctgctgaaaa cagtgcggct gatcaagttc ctgtaccaga gcaaccctcc tcccagcccc 780 gagggcacca gacaggcccg gagaaaccgg aggaggcggt ggagagagag gcagcggcag 840 atcagaagca tcagcgagtg gattctgagc acctacctgg gcagacccgc cgagcccgtg 900 cccctgcagc tgccccccct ggagagactg accctggact gcaacgagga ctgcggcacc 960 agcggcaccc agggagtggg cagcccccag atcctggtgg agagccctgc cgtgctggag 1020 agcggcacca aggagtgatg agcggccgc 1049
3-10	**Sequences**	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	AA
3-10-3	Length	341
3-10-4	Features	**REGION 1..341**
	Location/Qualifiers	note=Subtype B consensus Nef-Rev protein sequence construct **source 1..341** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-10-5	Residues	MDWTWILFLV AAATRVHSSK RSVVGWPTVR ERMRRAEPAA DGVGAVSRDL EKHGAITSSN 60 TAANNADCAW LEAQEEEEVG FPVRAQVALR AMTYKAAVDL SHFLKEKGGL EGLIYSQKRQ 120 DILDLWVYHT QGYFPDWQNY TPGPGIRYPL TFGWCFKLVP VEPEKVEEAN EGENNSAAHP 180 MSLHGMDDPE REVLVWKFDS RLAFHHMARE LHPEYYKDCR GRKRRSAGRS GDSDEELLKT 240 VRLIKFLYQS NPPPSPEGTR QARRNRRRRW RERQRQIRSI SEWILSTYLG RPAEPVPLQL 300 PPLERLTLDC NEDCGTSGTQ GVGSPQILVE SPAVLESGTK E 341
3-11	**Sequences**	
3-11-1	Sequence Number [ID]	11
3-11-2	Molecule Type	DNA
3-11-3	Length	1863
3-11-4	Features	**misc_feature 1..1863**
	Location/Qualifiers	note=Gag consensus DNA sequence of subtype A, B, C and D construct **source 1..1863** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-11-5	Residues	ggatccgcca ccatggactg gacctggatt ctgtttctgg tcgccgccgc cacaagagtg 60 cacagcggcg ccagagccag cgtgctgtcc ggcggcaagc tggacgcctg ggagaagatc 120 agactgaggc ctggcggcaa gaagaagtac cggctgaagc accttgtgtg ggccagcaga 180 gagctggaga gattcgccct gaatcctggc ctgctggaga ccagcgaggg ctgtaagcag 240 atcatcggcc agctgcagcc cgccctgcag accggcagcg aggagctgag aagcctgtac 300 aacaccgtgg ccaccctgta ctgcgtgcac gagaagatcg aggtgaagga caccaaggag 360 gccctggaca agatcgagga ggagcagaac aagagcaagc agaaggccca gcaggccgcc 420 gccgacaccg gcaacagcag ccaggtgtcc cagaactacc ccatcgtgca gaatctgcag 480 ggccagatgg tgcaccaggc catcagcccc agaaccctga atgcctgggt gaaggtgatc 540 gaggagaagg ccttcagccc tgaggtgatc cctatgttca gcgccctgag cgagggcgcc 600 acacctcagg acctgaacac catgctgaac acagtggggg gccaccaggc cgccatgcag 660 atgctgaagg ataccatcaa cgaggaggcc gccgagtggg acagactgca ccccgtgcac 720 gccggaccta tcgcccctgg ccagatgaga gagcccagag gcagcgacat cgccggcacc 780 acctccaccc tgcaagagca gatcggctgg atgaccagca acccccccat ccctgtgggc 840 gacatctaca agcggtggat catcctgggc ctgaacaaga ttgtgaggat gtacagcccc 900 gtgtccatcc tggatatcag gcagggcccc aaggagccct tcagagacta cgtggaccgg 960 ttcttcaaga ccctgagagc cgagcaggcc agccaggacg tgaagaactg gatgaccgag 1020 accctgctgg tgcagaacgc caaccccgac tgtaagacca tcctgagagc cctgggccct 1080 ggcgccaccc tggaggagat gatgaccgcc tgccagggag tgggcggacc cggccacaag 1140 gccagagtgc tggccgaggc catgagccag gccaccaaca gcaacatcat gatgcagcgg 1200 ggcaacttca gaggccccag gaggatcgtg aagtgcttca actgtggcaa ggagggccac 1260 atcgccagaa actgtagggc ccccaggaag aagggctgct ggaagtgtgg caaagagggg 1320 caccagatga aggactgtac cgagcggcag gccaatttcc tggggaagat ctggcccagc 1380 cacaagggca gacccggcaa tttcctgcag agcagacctg agcccaccgc ccctcccgcc 1440 gagagcttcg gcttcggcga ggagatcacc cccagcccca agcaggagcc caaggacaga 1500 gagctgtacc ctctggccag cctgaagagc ctgttcggca acgatcccct gagccagtac 1560 ccctacgacg tgcccgatta cgcctgagaa ttcgtaagta agtgtcatat gggagagctc 1620 gactagactg gacagccaat gacgggtaag agagtgacat ttctcactaa cctaagacag 1680 gagggccgtc aaagctactg cctaatccaa tgacgggtaa tagtgacaag aaatgtatca 1740 ctccaaccta agacaggcgc agcctccgag ggatgtgtct tttgtttttt ataattaaaa 1800 agggtgacat gtccggagcc gtgctgcccg gatgatgtct tggcctctgt ttgctgcggc 1860

		cgc 1863
3-12	**Sequences**	
3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	AA
3-12-3	Length	524
3-12-4	Features	**REGION 1..524**
	Location/Qualifiers	note=Gag consensus protein sequence of subtype A, B, C and D construc t
		source 1..524
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-12-5	Residues	MDWTWILFLV AAATRVHSGA RASVLSGGKL DAWEKIRLRP GGKKKYRLKH LVWASRELER 60 FALNPGLLET SEGCKQIIGQ LQPALQTGSE ELRSLYNTVA TLYCVHEKIE VKDTKEALDK 120 IEEEQNKSKQ KAQQAAADTG NSSQVSQNYP IVQNLQGQMV HQAISPRTLN AWVKVIEEKA 180 FSPEVIPMFS ALSEGATPQD LNTMLNTVGG HQAAMQMLKD TINEEAAEWD RLHPVHAGPI 240 APGQMREPRG SDIAGTTSTL QEQIGWMTSN PPIPVGDIYK RWIILGLNKI VRMYSPVSIL 300 DIRQGPKEPF RDYVDRFFKT LRAEQASQDV KNWMTETLLV QNANPDCKTI LRALGPGATL 360 EEMMTACQGV GGPGHKARVL AEAMSQATNS NIMMQRGNFR GPRRIVKCFN CGKEGHIARN 420 CRAPRKKGCW KCGKEGHQMK DCTERQANFL GKIWPSHKGR PGNFLQSRPE PTAPPAESFG 480 FGEEITPSPK QEPKDRELYP LASLKSLFGN DPLSQYPYDV PDYA 524
3-13	**Sequences**	
3-13-1	Sequence Number [ID]	13
3-13-2	Molecule Type	DNA
3-13-3	Length	43
3-13-4	Features	**misc_feature 1..43**
	Location/Qualifiers	note=IgE Primer Sequence 1
		source 1..43
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-13-5	Residues	gtcgctccgc tagcttgtgg gtcacagtct attatggggt acc 43
3-14	**Sequences**	
3-14-1	Sequence Number [ID]	14
3-14-2	Molecule Type	DNA
3-14-3	Length	35
3-14-4	Features	**misc_feature 1..35**
	Location/Qualifiers	note=IgE Primer Sequence 2
		source 1..35
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-14-5	Residues	ggtcggatcc ttactccacc actctccttt ttgcc 35
3-15	**Sequences**	
3-15-1	Sequence Number [ID]	15
3-15-2	Molecule Type	AA
3-15-3	Length	17
3-15-4	Features	**REGION 1..17**
	Location/Qualifiers	note=IgE leader sequence
		source 1..17
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-15-5	Residues	MDWTWILFLV AAATRVH 17
3-16	**Sequences**	
3-16-1	Sequence Number [ID]	16
3-16-2	Molecule Type	AA
3-16-3	Length	692
3-16-4	Features	**REGION 1..692**
	Location/Qualifiers	note=Subtype A consensus Envelope protein sequence
		source 1..692
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-16-5	Residues	SRVMGIQRNC QHLWRWGTMI LGMIIICSAA ENLWVTVYYG VPVWKDAETT LFCASDAKAY 60 DTEVHNVWAT HACVPTDPNP QEINLENVTE EFNMWKNNMV EQMHTDIISL WDQSLKPCVK 120 LTPLCVTLNC SNVNVTTNIM KGEIKNCSFN MTTELRDKKQ KVYSLFYKLD VVQINKSNSS 180 SQYRLINCNT SAITQACPKV SFEPIPIHYC APAGFAILKC KDKEFNGTGP CKNVSTVQCT 240 HGIKPVVSTQ LLLNGSLAEE EVMIRSENIT NNAKNIIVQL TKPVKINCTR PNNNTRKSIR 300 IGPGQAFYAT GDIIGDIRQA HCNVSRTEWN ETLQKVAKQL RKYFNNKTII FTNSSGGRLR 360 ITTHSFNCGG EFFYCNTSGL FNSTWNGNGT KKKNSTESND TITLPCRIKQ IINMWQRVGQ 420

		AMYAPPIQGV IRCESNITGL LLTRDGGDNN SKNETFRPGG GDMRDNWRSE LYKYKVVKIE 480 PLGVAPTKAK RRVVEREKRA VGIGAVFLGF LGAAGSTMGA ASITLTVQAR QLLSGIVQQQ 540 SNLLRAIEAQ QHLLKLTVWG IKQLQARVLA VERYLKDQQL LGIWGCSGKL ICTTNVPWNS 600 SWSNKSQSEI WDNMTWLQWD KEISNYTDII YNLIEESQNQ QEKNEQDLLA LDKWANLWNW 660 FDISNWLWYI KIFIMIVGGL IGLRIVFAVL SV 692
3-17	**Sequences**	
3-17-1	Sequence Number [ID]	17
3-17-2	Molecule Type	AA
3-17-3	Length	697
3-17-4	Features	**REGION 1..697**
	Location/Qualifiers	note=Subtype B consensus Envelope protein sequence **source 1..697** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-17-5	Residues	RVKGIRKNYQ HLWRWGTMLL GMLMICSAAE KLWVTVYYGV PVWKEATTTL FCASDAKAYD 60 TEVHNVWATH ACVPTDPNPQ EVVLENVTEN FNMWKNNMVE QMHEDIISLW DQSLKPCVKL 120 TPLCVTLNCT DLSGEKMEKG EIKNCSFNIT TSIRDKVQKE YALFYKLDVV PIDNDNTSYR 180 LISCNTSVIT QACPKVSFEP IPIHYCAPAG FAILKCNDKK FNGTGPCTNV STVQCTHGIR 240 PVVSTQLLLN GSLAEEEVVI RSENFTNNAK TIIVQLNESV EINCTRPNNN TRKSIHIGPG 300 QAFYTTGEII GDIRQAHCNI SRAKWNNTLK QIVKKLREQF GNKTIVFNQS SGGRPRIVMH 360 SFNCGGEFFY CNTTQLFNST WNVNGTWNNN TEGNDTITLP CRIKQIINMW QEVGKAMYAP 420 PIRGQIRCSS NITGLLLTRD GGNNNTNETE IFRPGGGDMR DNWRSELYKY KVVKIEPLGV 480 APTKAKRRVV QREKRAVGIG AMFLGFLGAA GSTMGAASMT LTVQARQLLS GIVQQQNNLL 540 RAIEAQQHLL QLTVWGIKQL QARVLAVERY LKDQQLLGIW GCSGKLICTT TVPWNASWSN 600 KSLDEIWDNM TWMEWEREID NYTSLIYTLI EESQNQQEKN EQELLELDKW ASLWNWFDIT 660 NWLWYIKIFI MIVGGLIGLR IVFAVLSIYP YDVPDYA 697
3-18	**Sequences**	
3-18-1	Sequence Number [ID]	18
3-18-2	Molecule Type	AA
3-18-3	Length	687
3-18-4	Features	**REGION 1..687**
	Location/Qualifiers	note=Subtype C consensus Envelope protein sequence **source 1..687** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-18-5	Residues	RVRGILRNCQ QWWIWGILGF WMLMICNVMG NLWVTVYYGV PVWKEAKTTL FCASDAKAYE 60 TEVHNVWATH ACVPTDPNPQ EMVLENVTEN FNMWKNDMVD QMHEDIISLW DQSLKPCVKL 120 TPLCVTLNCR NNVNNNNTMK EEIKNCSFNI TTELRDKKQK VYALFYRLDI VPLNEKNNSN 180 DYRLINCNTS AITQACPKVS FDPIPIHYCA PAGYAILKCN NKTFNGTGPC NNVSTVQCTH 240 GIKPVVSTQL LLNGSLAEEE IIIRSENLTN NAKTIIVHLN ESVEIVCTRP NNNTRKSIRI 300 GPGQTFYATG DIIGDIRQAH CNISEEKWNK TLQRVSEKLK EHFPNKTIKF APSSGGRLEI 360 TTHSFNCRGE FFYCNTSKLF NSTYMPNSTN NTNTTITLPC RIKQIINMWQ EVGRAMYAPP 420 IEGNITCKSN ITGLLLTRDG GKNDTNDTET FRPGGGDMRD NWRSELYKYK VVEIKPLGVA 480 PTKAKRRVVE REKRAVGIGA VFLGFLGAAG STMGAASITL TVQARQLLSG IVQQQSNLLR 540 AIEAQQHMLQ LTVWGIKQLQ TRVLAIERYL KDQQLLGIWG CSGKLICTTA VPWNSSWSNK 600 SQEDIWDNMT WMQWDREISN YTDTIYRLLE DSQNQQEKNE KDLLALDSWK NLWNWFDITN 660 WLWYIKIFIM IVGGLIGLRI IFAVLSI 687
3-19	**Sequences**	
3-19-1	Sequence Number [ID]	19
3-19-2	Molecule Type	AA
3-19-3	Length	696
3-19-4	Features	**REGION 1..696**
	Location/Qualifiers	note=Subtype D consensus Envelope protein sequence **source 1..696** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-19-5	Residues	RVRGIKRNYQ HLWKWGTMLL GMLMTCSVAE NLWVTVYYGV PVWKEATTTL FCASDAKSYK 60 TEAHNIWATH ACVPTDPNPQ EIELENVTEN FNMWKNNMVE QMHEDIISLW DQSLKPCVKL 120 TPLCVTLNCT DGMRNDTNDT NVTMEEGEMK NCSFNITTEV RDKKKQVHAL FYKLDVVPID 180 DNNTNNSNYR LINCNTSAIT QACPKVTFEP IPIHYCAPAG FAILKCKDKK FNGTGPCKNV 240 STVQCTHGIR PVVSTQLLLN GSLAEEEIII RSENLTNNAK IIIVQLNESV TINCTRPYNN 300 TRKRIPIGLG QAFYTTRGII GDIRQAHCNI SGAEWNKTLQ QVAKKLGDLL NKTTIIFKPS 360 SGGRPRITTH SFNCGGEFFY CNTSRLFNST WSKNSTSNST KENNTITLPC RIKQIINMWQ 420 GVGKAMYAPP IEGLIKCSSN ITGLLLTRDG GANNSHNETF RPGGGDMRDN WRSELYKYKV 480 VKIEPLGVAP TRAKRRVVER EKRAIGLGAM FLGFLGAAGS TMGAASLTLT VQARQLLSGI 540 VQQQNNLLRA IEAQQHLLQL TVWGIKQLQA RILAVERYLK DQQLLGIWGC SGKHICTTTV 600 PWNSSWSNKS LDEIWNNMTW MEWEREIDNY TGLIYSLIEE SQTQQEKNEQ ELLELDKWAS 660 LWNWFSITQW LWYIKIFIMI VGGLIGLRIV FAVLSL 696
3-20	**Sequences**	

3-20-1	Sequence Number [ID]	20
3-20-2	Molecule Type	AA
3-20-3	Length	323
3-20-4	Features	**REGION 1..323**
	Location/Qualifiers	note=Subtype B consensus Nef-Rev protein sequence
		source 1..323
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-20-5	Residues	SKRSVVGWPT VRERMRRAEP AADGVGAVSR DLEKHGAITS SNTAANNADC AWLEAQEEEE 60 VGFPVRAQVA LRAMTYKAAV DLSHFLKEKG GLEGLIYSQK RQDILDLWVY HTQGYFPDWQ 120 NYTPGPGIRY PLTFGWCFKL VPVEPEKVEE ANEGENNSAA HPMSLHGMDD PEREVLVWKF 180 DSRLAFHHMA RELHPEYYKD CRGRKRRSAG RSGDSDEELL KTVRLIKFLY QSNPPPSPEG 240 TRQARRNRRR RWRERQRQIR SISEWILSTY LGRPAEPVPL QLPPLERLTL DCNEDCGTSG 300 TQGVGSPQIL VESPAVLESG TKE 323
3-21	**Sequences**	
3-21-1	Sequence Number [ID]	21
3-21-2	Molecule Type	AA
3-21-3	Length	506
3-21-4	Features	**REGION 1..506**
	Location/Qualifiers	note=Gag consensus protein sequence of subtype A, B, C and D
		source 1..506
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-21-5	Residues	GARASVLSGG KLDAWEKIRL RPGGKKKYRL KHLVWASREL ERFALNPGLL ETSEGCKQII 60 GQLQPALQTG SEELRSLYNT VATLYCVHEK IEVKDTKEAL DKIEEEQNKS KQKAQQAAAD 120 TGNSSQVSQN YPIVQNLQGQ MVHQAISPRT LNAWVKVIEE KAFSPEVIPM FSALSEGATP 180 QDLNTMLNTV GGHQAAMQML KDTINEEAAE WDRLHPVHAG PIAPGQMREP RGSDIAGTTS 240 TLQEQIGWMT SNPPIPVGDI YKRWIILGLN KIVRMYSPVS ILDIRQGPKE PFRDYVDRFF 300 KTLRAEQASQ DVKNWMTETL LVQNANPDCK TILRALGPGA TLEEMMTACQ GVGGPGHKAR 360 VLAEAMSQAT NSNIMMQRGN FRGPRRIVKC FNCGKEGHIA RNCRAPRKKG CWKCGKEGHQ 420 MKDCTERQAN FLGKIWPSHK GRPGNFLQSR PEPTAPPAES FGFGEEITPS PKQEPKDREL 480 YPLASLKSLF GNDPLSQYPY DVPDYA 506
3-22	**Sequences**	
3-22-1	Sequence Number [ID]	22
3-22-2	Molecule Type	DNA
3-22-3	Length	818
3-22-4	Features	**misc_feature 1..818**
	Location/Qualifiers	note=HPV genotype 16 E6-E7 DNA sequence
		source 1..818
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-22-5	Residues	gaattcgcca ccatggactg gacctggatc ctgttcctgg tggccgccgc cacacgggtg 60 cacagcttcc aggaccccca ggagagcggc agaaagctgc ctcagctgtg taccgagctg 120 cagaccacca tccacgacat catcctggag tgtgtgtact gtaagcagca gctgctgagg 180 agagaggtgt acgaccggga cctgtgtatc gtgtacaggg acggcaatcc ctacgccgtg 240 tgtgacaagt gcctgaagtt ctacagcaag atcagcgagt accggcacta ctgctacagc 300 ctgtacggca ccaccctgga gcagcagtac aacaagcccc tgtgtgacct gctgatccgg 360 tgtatcaact gccagaagcc cctgcagaga cacctggaca agaagcagcg gttccacaac 420 atcaggggca gatggaccgg cagatgtatg agctgctgcc ggagcagcag aaccagaagg 480 gagacccagc tgagaggccg gaagagaaga agccacggcg atacccccac cctgcacgag 540 tacatgctgg acctgcagcc tgagaccacc gatctgtacg gctacggcca gctgaatgac 600 agcagcgagg aggaggatga gatcgacggc cctgccggcc aggccgagcc cgacagagcc 660 cactacaaca tcgtgacctt ttgctgtaag tgtgacagca ccctgagact gtgcgtgcag 720 agcacccacg tggacatcag aaccctggag gatctgctga tgggcaccct gggcatcgtg 780 tgtcccatct gctcccagaa accctgatga gcggccgc 818
3-23	**Sequences**	
3-23-1	Sequence Number [ID]	23
3-23-2	Molecule Type	AA
3-23-3	Length	264
3-23-4	Features	**REGION 1..264**
	Location/Qualifiers	note=HPV genotype 16 E6-E7 protein sequence
		source 1..264
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-23-5	Residues	MDWTWILFLV AAATRVHSFQ DPQESGRKLP QLCTELQTTI HDIILECVYC KQQLLRREVY 60 DRDLCIVYRD GNPYAVCDKC LKFYSKISEY RHYCYSLYGT TLEQQYNKPL CDLLIRCINC 120 QKPLQRHLDK KQRFHNIRGR WTGRCMSCCR SSRTRRETQL RGRKRRSHGD TPTLHEYMLD 180

		LQPETTDLYG YGQLNDSSEE EDEIDGPAGQ AEPDRAHYNI VTFCCKCDST LRLCVQSTHV 240 DIRTLEDLLM GTLGIVCPIC SQKP 264
3-24	**Sequences**	
3-24-1	Sequence Number [ID]	24
3-24-2	Molecule Type	AA
3-24-3	Length	15
3-24-4	Features	**REGION 1..15**
	Location/Qualifiers	note=HPV E6 immunodominant epitope
		source 1..15
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-24-5	Residues	LCIVYRDGNP YAVCD 15
3-25	**Sequences**	
3-25-1	Sequence Number [ID]	25
3-25-2	Molecule Type	AA
3-25-3	Length	15
3-25-4	Features	**REGION 1..15**
	Location/Qualifiers	note=HPV E7 immunodominant epitope
		source 1..15
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-25-5	Residues	AEPDRAHYNI VTFCC 15
3-26	**Sequences**	
3-26-1	Sequence Number [ID]	26
3-26-2	Molecule Type	AA
3-26-3	Length	142
3-26-4	Features	**REGION 1..142**
	Location/Qualifiers	note=HPV E6 consensus sequence
		source 1..142
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-26-5	Residues	FQDPQESGRK LPQLCTELQT TIHDIILECV YCKQQLLRRE VYDRDLCIVY RDGNPYAVCD 60 KCLKFYSKIS EYRHYCYSLY GTTLEQQYNK PLCDLLIRCI NCQKPLQRHL DKKQRFHNIR 120 GRWTGRCMSC CRSSRTRRET QL 142
3-27	**Sequences**	
3-27-1	Sequence Number [ID]	27
3-27-2	Molecule Type	AA
3-27-3	Length	97
3-27-4	Features	**REGION 1..97**
	Location/Qualifiers	note=HPV E7 consensus sequence
		source 1..97
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-27-5	Residues	HGDTPTLHEY MLDLQPETTD LYGYGQLNDS SEEEDEIDGP AGQAEPDRAH YNIVTFCCKC 60 DSTLRLCVQS THVDIRTLED LLMGTLGIVC PICSQKP 97
3-28	**Sequences**	
3-28-1	Sequence Number [ID]	28
3-28-2	Molecule Type	AA
3-28-3	Length	18
3-28-4	Features	**REGION 1..18**
	Location/Qualifiers	note=IgE Leader Sequence
		source 1..18
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-28-5	Residues	MDWTWILFLV AAATRVHS 18
3-29	**Sequences**	
3-29-1	Sequence Number [ID]	29
3-29-2	Molecule Type	AA
3-29-3	Length	7
3-29-4	Features	**REGION 1..7**
	Location/Qualifiers	note=Proteolytic Cleavage Sequence
		source 1..7
		mol_type=protein
		organism=synthetic construct

	NonEnglishQualifier Value	
3-29-5	Residues	RGRKRRS 7
3-30	**Sequences**	
3-30-1	Sequence Number [ID]	30
3-30-2	Molecule Type	DNA
3-30-3	Length	1766
3-30-4	Features	**misc_feature 1..1766**
	Location/Qualifiers	note=HCV genotype 1a and 1b consensus E1-E2 DNA sequence
		source 1..1766
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-30-5	Residues	gaattcgcca ccatggactg gacctggatc ctgttcctgg tggccgctgc aacacgggtg 60 cacagctacc aagtgaggaa tagcagcggc ctgtaccacg tgaccaacga ctgctccaac 120 agcagcatcg tgtacgaggc cgccgacatg atcatgcaca cccccggctg tgtgccctgt 180 gtgagagagg gcaacagctc cagatgctgg gtggccctga cccctaccgt ggccgccaga 240 gatggcagcc tgcccaccac caccctgagg agacacgtgg acctgcttgt gggcagcgcc 300 accctgtgta gcgccatgta tgtgggcgat ctgtgtggca gcgtgtttct tgtgggccag 360 ctgttcacct tcagccccag aaggcactgg accgtgcagg actgtaactg ctccatctac 420 cccggccaca tcaccggcca cagaatggcc tgggacatga tgatgaactg gagccctacc 480 accgccctgg tggtgtccca gctgctgaga atccctcagg ccatcgtgga catggtggcc 540 ggagcccact ggggcgtgct ggccggcatc gcctacttca gcatggtggg caactgggcc 600 aaggtgctcg tggtgctgct gctgttcgcc ggcgtggacg gcagaggcag gaagagaagg 660 agcgagaccc acgtgaccgg cggcaccgcc ggcagaacca cagccggcct tgtgggcctg 720 ttcacccctg gcgccaagca gaacatccag ctgatcaaca ccaacggcag ctggcacatc 780 aacagcaccg ccctgaactg taacgacagc ctgaacaccg gctggctggc cggcctgttc 840 taccagcaca agttcaacag cagcggctgc cccgagagaa tggccagctg tagacccctg 900 gatgagttcg cccagggctg gggccccatc acctacgcca atggcagcgg ccctgaccag 960 agaccctact gctggcacta cgcccccaga ccctgtggca tcgtgcccgc caagagcgtg 1020 tgtggccccg tgtactgctt cacccctagc cccgtggttg tgggcaccac cgacagaagc 1080 ggagccccca cctacagctg gggcgagaac gagaccgacg tgctggtgct gaacaacacc 1140 agaccccccc tgggcaattg gttcggctgt acctggatga acagcaccgg cttcaccaaa 1200 gtgtgtggcg cccctccctg tgtgatcggc ggagtgggca acaacaccct gacctgcccc 1260 accgactgct tcagaaagca ccccgaggcc acctactcca gatgtggcag cggaccttgg 1320 ctgaccccca gatgtatggt ggactacccc tacaggctgt ggcactaccc ctgtaccgtg 1380 aacttcacca tcttcaaagt gaggatgtat gtgggggggcg tggagcacag actggaggcc 1440 gcctgtaatt ggaccagggg cgagagatgt gacctggagg accgggatag aagcgagctg 1500 tcccctctgc tgctgtccac caccgagtgg caggtgctgc cttgtagctt caccaccctg 1560 cccgccctga gcaccggcct gatccacctg caccagaaca tcgtggacgt gcagtacctg 1620 tacggagtgg gctctagcat cgtgtcctgg gccatcaagt gggagtacgt ggtgctgctg 1680 ttcctgctgc tggccgacgc cagagtgtgt agctgcctgt ggatgatgct gctgatcagc 1740 caggccgagg cctgatgagc ggccgc 1766
3-31	**Sequences**	
3-31-1	Sequence Number [ID]	31
3-31-2	Molecule Type	AA
3-31-3	Length	580
3-31-4	Features	**REGION 1..580**
	Location/Qualifiers	note=HCV genotype 1a and 1b consensus E1-E2 protein sequence
		source 1..580
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-31-5	Residues	MDWTWILFLV AAATRVHSYQ VRNSSGLYHV TNDCSNSSIV YEAADMIMHT PGCVPCVREG 60 NSSRCWVALT PTVAARDGSL PTTTLRRHVD LLVGSATLCS AMYVGDLCGS VFLVGQLFTF 120 SPRRHWTVQD CNCSIYPGHI TGHRMAWDMM MNWSPTTALV VSQLLRIPQA IVDMVAGAHW 180 GVLAGIAYFS MVGNWAKVLV VLLLFAGVDG RGRKRRSETH VTGGTAGRTT AGLVGLFTPG 240 AKQNIQLINT NGSWHINSTA LNCNDSLNTG WLAGLFYQHK FNSSGCPERM ASCRPLDEFA 300 QGWGPITYAN GSGPDQRPYC WHYAPRPCGI VPAKSVCGPV YCFTPSPVVV GTTDRSGAPT 360 YSWGENETDV LVLNNTRPPL GNWFGCTWMN STGFTKVCGA PPCVIGGVGN NTLTCPTDCF 420 RKHPEATYSR CGSGPWLTPR CMVDYPYRLW HYPCTVNFTI FKVRMYVGGV EHRLEAACNW 480 TRGERCDLED RDRSELSPLL LSTTEWQVLP CSFTTLPALS TGLIHLHQNI VDVQYLYGVG 540 SSIVSWAIKW EYVVLLFLLL ADARVCSCLW MMLLISQAEA 580
3-32	**Sequences**	
3-32-1	Sequence Number [ID]	32
3-32-2	Molecule Type	AA
3-32-3	Length	192
3-32-4	Features	**REGION 1..192**
	Location/Qualifiers	note=HCV E1 consensus sequence
		source 1..192
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	

3-32-5	Residues	YQVRNSSGLY HVTNDCSNSS IVYEAADMIM HTPGCVPCVR EGNSSRCWVA LTPTVAARDG 60 SLPTTTLRRH VDLLVGSATL CSAMYVGDLC GSVFLVGQLF TFSPRRHWTV QDCNCSIYPG 120 HITGHRMAWD MMMNWSPTTA LVVSQLLRIP QAIVDMVAGA HWGVLAGIAY FSMVGNWAKV 180 LVVLLLFAGV DG 192
3-33	**Sequences**	
3-33-1	Sequence Number [ID]	33
3-33-2	Molecule Type	AA
3-33-3	Length	363
3-33-4	Features	**REGION 1..363**
	Location/Qualifiers	note=HCV E2 consensus sequence **source 1..363** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-33-5	Residues	ETHVTGGTAG RTTAGLVGLF TPGAKQNIQL INTNGSWHIN STALNCNDSL NTGWLAGLFY 60 QHKFNSSGCP ERMASCRPLD EFAQGWGPIT YANGSGPDQR PYCWHYAPRP CGIVPAKSVC 120 GPVYCFTPSP VVVGTTDRSG APTYSWGENE TDVLVLNNTR PPLGNWFGCT WMNSTGFTKV 180 CGAPPCVIGG VGNNTLTCPT DCFRKHPEAT YSRCGSGPWL TPRCMVDYPY RLWHYPCTVN 240 FTIFKVRMYV GGVEHRLEAA CNWTRGERCD LEDRDRSELS PLLLSTTEWQ VLPCSFTTLP 300 ALSTGLIHLH QNIVDVQYLY GVGSSIVSWA IKWEYVVLLF LLLADARVCS CLWMMLLISQ 360 AEA 363
3-34	**Sequences**	
3-34-1	Sequence Number [ID]	34
3-34-2	Molecule Type	DNA
3-34-3	Length	3512
3-34-4	Features	**source 1..3512**
	Location/Qualifiers	mol_type=other DNA organism=Homo sapiens
	NonEnglishQualifier Value	
3-34-5	Residues	ggtaccgaat tcgccaccat ggactggacc tggatcctgt tcctggtggc cgctgccaca 60 agagtgcaca gccccagggc ccccaggtgc agagccgtgc ggagcctgct gcggagccac 120 taccgggagg tgctgcccct ggccaccttc gtgcggaggc tgggccctca ggggtggcgg 180 ctggtgcaga gaggcgaccc tgccgccttc agagccctgg tggcccagtg cctggtgtgc 240 gtgccctggg acgccagacc tccccctgcc gcccctagct tccggcaggt gtcctgcctg 300 aaagaactgg tggcccgggt gctgcagcgg ctgtgcgaga ggggcgccaa gaacgtgctg 360 gccttcggct tcgccctgct ggacggcgcc agaggcggcc ctcccgaggc cttcaccacc 420 tccgtgagaa gctacctgcc caacaccgtg accgacgccc tgagaggcag cggcgcttgg 480 ggcctgctgc tgcgcagagt gggcgacgac gtgctggtgc acctgctggc cagatgcgcc 540 ctgttcgtgc tggtcgcccc cagctgcgcc taccaggtgt gcggcccacc cctgtaccag 600 ctgggagccg ccacccaggc cagacccccc cctcacgcct ccggccccag gcggagactg 660 ggctgcgagc gggcctggaa ccacagcgtg cgggaggccg gcgtgcccct gggcctgcca 720 gcccctggcg ccagaagaag gggcggcagc gccagcagaa gcctgccccc gcccaagcgg 780 cccagacgcg gagccgcccc tgagcccgag agaaccccg tgggccaggg ctcttgggcc 840 caccctggcc ggaccagagg ccccagcgac cggggcttct gcgtggtgtc ccccgccaga 900 cccgccgagg aagccacctc cctggaaggc gccctgagcg gcaccaggca cagccacccc 960 agcgtgggcc gccagcacca cgccggaccc cccagcacct ccaggccccc caggccctgg 1020 gacacccctt gccccccctgt gtacgccgag accaagcact tcctgtacag cagcggcgac 1080 aaagagcagc tgcggcccag cttcctgctg tccagcctga ggccctccct gaccggcgct 1140 aggcgcctgg tggagaccat ctttctgggc agccggccct ggatgcccgg caccccagg 1200 cggctgccca ggctgcccca gcggtactgg cagatgaggc ctctgttcct ggaactgctg 1260 ggcaaccacg cccagtgccc ctacggcgtg ctgctgaaaa cccactgccc cctgagagcc 1320 gccgtgaccc cagccgccgg agtgtgcgcc agagagaagc ctcagggcag cgtggccgct 1380 cccgaggaag aggacaccga ccccagacgc ctggtgcagc tgctgcggca gcacagcagc 1440 ccttggcagg tgtacggctt cgtgcgggcc tgcctgagaa ggctggtgcc ccctggcctg 1500 tggggcagca ggcacaacga gcggcggttt ctgcggaaca ccaagaagtt catcagcctg 1560 gggaagcacg ccaagctgtc cctgcaggaa ctgacctgga agatgagcgt gcggggctgc 1620 gcctggctga gaagatcccc tggcgtgggc tgcgtgcctg ccgccgagca ccggctgcgg 1680 gaggaaatcc tggccaagtt cctgcactgg ctgatgagcg tgtacgtggt ggagctgctg 1740 agatccttct tctacgtgac cgagaccacc ttccagaaga actacctgtt cttctaccgg 1800 aagagcgtgt ggagcaagct gcagagcatc ggcatccggc agcacctgaa gcgggtgcag 1860 ctgagagagc tgtccgaggc cgaagtgagg cagcaccggg aggccagacc tgccctgctg 1920 accagccggc tgcggttcat ccccaagccc gacggcctgc ggcccatcgt gaacatggac 1980 tacgtggtgg gcgccaggac cttccggcgg gagaagcggg ccgagcggct gacctcgagg 2040 gtgaaggccc tgttcagcgt gctgaactac gagcgggcca ggcggccagg cctgctgggc 2100 gccagcgtgc tgggcctgga cgacatccac cgggcctggc ggaccttcgt gctgagagtg 2160 cgggcccagg acccccctcc cgagctgtac ttcgtgaagg tggacgtgac aggcgcctac 2220 gacaccatcc cccaggaccg gctgaccgag gtgatcgcca gcatcatcaa gccccagaac 2280 acctactgcg tgcggagata cgccgtggtg cagaaggccg cccacggcca cgtgcggaag 2340 gccttcaaga gccacgtgag caccctgacc gacctgcagc cctacatgcg gcagttcgtg 2400 gcccacctgc aggaaaccag ccccctgcgg gatgccgtgg tgatcgagca gagcagcagc 2460 ctgaacgagg ccagcagcgg cctgttcgac gtgttcctga gattcatgtg ccaccacgcc 2520 gtgcggatcc ggggcaagag ctacgtgcag tgccagggca tcccacaggg cagcatcctg 2580 tccaccctgc tgtgctccct gtgctacggc gacatggaaa acaagctgtt cgccggcatc 2640 aggcgggacg gactgctgct gagactggtg gacgacttcc tgctggtgac cccccacctg 2700

		acccacgcca agacctttct gcggaccctg gtgcgcggcg tgcccgagta cggctgcgtg 2760 gtgaacctga gaaagaccgt ggtgaacttc cccgtggagg acgaggccct gggcggcaca 2820 gccttcgtgc agatgcctgc ccatggactg ttcccttggt gcgggctgct gctggacacc 2880 cggaccctgg aagtgcagag cgactacagc agctacgccc ggaccagcat ccgggcctcc 2940 ctgaccttca acaggggctt caaggccggc aggaacatgc ggcggaagct gtttggcgtg 3000 ctgcggctga agtgccacag cctgtttctg tacctgcagg tgaacagcct gcagaccgtg 3060 tgcaccaaca tctacaagat cctgctgctg caggcctacc ggttccacgc ctgcgtgctg 3120 cagctgccct ttcaccagca ggtgtggaag aaccctacct tcttcctgcg ggtgatcagc 3180 gacaccgcca gcctgtgcta cagcatcctg aaggccaaga acgccggcat gagcctgggc 3240 gccaagggag ccgccggacc tctgcccagc gaggccgtgc agtggctgtg ccaccaggcc 3300 tttctgctga agctgacccg gcaccgggtg acctacgtgc ccctgctggg cagcctgcgg 3360 accgcccaga cccagctgtc ccggaagctg cctggcacca ccctgacagc cctggaagcc 3420 gccgccaacc ccgccctgcc ctccgacttc aagaccatcc tggactaccc ctacgacgtg 3480 cccgactacg cctgatgagc ggccgcgagc tc 3512
3-35	**Sequences**	
3-35-1	Sequence Number [ID]	35
3-35-2	Molecule Type	AA
3-35-3	Length	1158
3-35-4	Features	**source 1..1158**
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	
3-35-5	Residues	MDWTWILFLV AAATRVHSPR APRCRAVRSL LRSHYREVLP LATFVRRLGP QGWRLVQRGD 60 PAAFRALVAQ CLVCVPWDAR PPPAAPSFRQ VSCLKELVAR VLQRLCERGA KNVLAFGFAL 120 LDGARGGPPE AFTTSVRSYL PNTVTDALRG SGAWGLLLRR VGDDVLVHLL ARCALFVLVA 180 PSCAYQVCGP PLYQLGAATQ ARPPPHASGP RRRLGCERAW NHSVREAGVP LGLPAPGARR 240 RGGSASRSLP LPKRPRRGAA PEPERTPVGQ GSWAHPGRTR GPSDRGFCVV SPARPAEEAT 300 SLEGALSGTR HSHPSVGRQH HAGPPSTSRP PRPWDTPCPP VYAETKHFLY SSGDKEQLRP 360 SFLLSSLRPS LTGARRLVET IFLGSRPWMP GTPRRLPRLP QRYWQMRPLF LELLGNHAQC 420 PYGVLLKTHC PLRAAVTPAA GVCAREKPQG SVAAPEEEDT DPRRLVQLLR QHSSPWQVYG 480 FVRACLRRLV PPGLWGSRHN ERRFLRNTKK FISLGKHAKL SLQELTWKMS VRGCAWLRRS 540 PGVGCVPAAE HRLREEILAK FLHWLMSVYV VELLRSFFYV TETTFQKNYL FFYRKSVWSK 600 LQSIGIRQHL KRVQLRELSE AEVRQHREAR PALLTSRLRF IPKPDGLRPI VNMDYVVGAR 660 TFRREKRAER LTSRVKALFS VLNYERARRP GLLGASVLGL DDIHRAWRTF VLRVRAQDPP 720 PELYFVKVDV TGAYDTIPQD RLTEVIASII KPQNTYCVRR YAVVQKAAHG HVRKAFKSHV 780 STLTDLQPYM RQFVAHLQET SPLRDAVVIE QSSSLNEASS GLFDVFLRFM CHHAVRIRGK 840 SYVQCQGIPQ GSILSTLLCS LCYGDMENKL FAGIRRDGLL LRLVDDFLLV TPHLTHAKTF 900 LRTLVRGVPE YGCVVNLRKT VVNFPVEDEA LGGTAFVQMP AHGLFPWCGL LLDTRTLEVQ 960 SDYSSYARTS IRASLTFNRG FKAGRNMRRK LFGVLRLKCH SLFLYLQVNS LQTVCTNIYK 1020 ILLLQAYRFH ACVLQLPFHQ QVWKNPTFFL RVISDTASLC YSILKAKNAG MSLGAKGAAG 1080 PLPSEAVQWL CHQAFLLKLT RHRVTYVPLL GSLRTAQTQL SRKLPGTTLT ALEAAANPAL 1140 PSDFKTILDY PYDVPDYA 1158
3-36	**Sequences**	
3-36-1	Sequence Number [ID]	36
3-36-2	Molecule Type	DNA
3-36-3	Length	1707
3-36-4	Features	**misc_feature 1..1707**
	Location/Qualifiers	note=Influenza H5N1 HA consensus sequence **source 1..1707** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-36-5	Residues	atggaaaaga tcgtgctgct gttcgccatc gtgagcctgg tgaagagcga ccagatctgc 60 atcggctacc acgccaacaa cagcaccgag caggtggaca ccatcatgga aaaaaacgtg 120 accgtgaccc acgcccagga catcctggaa aagacccaca acggcaagct gtgcgacctg 180 gacggcgtga agcccctgat cctgcgggac tgcagcgtgg ccggctggct gctgggcaac 240 cccatgtgcg acgagttcat caacgtgccc gagtggagct acatcgtgga gaaggccaac 300 cccgtgaacg acctgtgcta ccccggcgac ttcaacgact acgaggaact gaagcacctg 360 ctgtcccgga tcaaccactt cgagaagatc cagatcatcc ccaagagcag ctggtccagc 420 cacgaggcca gcctgggcgt gagcagcgcc tgcccatacc agggcaagtc cagcttcttc 480 cggaacgtgg tgtggctgat caagaagaac agcacctacc ccaccatcaa gcggagctac 540 aacaacacca accaggaaga tctgctggtc ctgtggggca tccaccaccc caacgacgcc 600 gccgagcaga ccaagctgta ccagaacccc accacctaca tcagcgtggg caccagcacc 660 ctgaaccagc ggctggtgcc ccggatcgcc acccggtcca aggtgaacgg ccagagcggc 720 cggatggaat tcttctggac catcctgaag cccaacgatg ccatcaactt cgagagcaac 780 ggcaacttca tcgcccccga gtacgcctac aagatcgtga agaagggcga cagcaccatc 840 atgaagagcg agctggaata cggcaactgc aacaccaagt gccagacccc catgggcgcc 900 atcaacagca gcatgccctt ccacaacatc cacccctga ccatcggcga gtgccccaag 960 tacgtgaaga gcaacaggct ggtgctggcc accggcctgc ggaacagccc ccagcgggag 1020 cggcgggccg ccgcccgggg cctgttcggc gccatcgccg gcttcatcga gggcggctgg 1080 cagggcatgg tggacgggtg gtacggctac caccacagca atgagcaggg cagcggctac 1140 gccgccgaca aagagagcac ccagaaggcc atcgacggcg tcaccaacaa ggtgaacagc 1200 atcatcgaca agatgaacac ccagttcgag gccgtgggcc gggagttcaa caacctggaa 1260 cggcggatcg agaacctgaa caagaaaatg gaagatggct tcctggacgt gtggacctac 1320

		aacgccgagc tgctggtgct gatggaaaac gagcggaccc tggacttcca cgacagcaac 1380 gtgaagaacc tgtacgacaa agtgcggctg cagctgcggg acaacgccaa agagctgggc 1440 aacggctgct tcgagttcta ccacaagtgc gacaacgagt gcatggaaag cgtgcggaac 1500 ggcacctacg actaccccca gtacagcgag gaagcccggc tgaagcggga ggaaatcagc 1560 ggcgtgaaac tggaaagcat cggcatctac cagatcctga gcatctacag caccgtggcc 1620 agcagcctgg ccctggccat catggtggcc ggcctgagcc tgtggatgtg cagcaacggc 1680 agcctgcagt gccggatctg catctag 1707
3-37	**Sequences**	
3-37-1	Sequence Number [ID]	37
3-37-2	Molecule Type	AA
3-37-3	Length	568
3-37-4	Features	**REGION 1..568**
	Location/Qualifiers	note=Influenza H5N1 HA consensus sequence **source 1..568** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-37-5	Residues	MEKIVLLFAI VSLVKSDQIC IGYHANNSTE QVDTIMEKNV TVTHAQDILE KTHNGKLCDL 60 DGVKPLILRD CSVAGWLLGN PMCDEFINVP EWSYIVEKAN PVNDLCYPGD FNDYEELKHL 120 LSRINHFEKI QIIPKSSWSS HEASLGVSSA CPYQGKSSFF RNVVWLIKKN STYPTIKRSY 180 NNTNQEDLLV LWGIHHPNDA AEQTKLYQNP TTYISVGTST LNQRLVPRIA TRSKVNGQSG 240 RMEFFWTILK PNDAINFESN GNFIAPEYAY KIVKKGDSTI MKSELEYGNC NTKCQTPMGA 300 INSSMPFHNI HPLTIGECPK YVKSNRLVLA TGLRNSPQRE RRAAARGLFG AIAGFIEGGW 360 QGMVDGWYGY HHSNEQGSGY AADKESTQKA IDGVTNKVNS IIDKMNTQFE AVGREFNNLE 420 RRIENLNKKM EDGFLDVWTY NAELLVLMEN ERTLDFHDSN VKNLYDKVRL QLRDNAKELG 480 NGCFEFYHKC DNECMESVRN GTYDYPQYSE EARLKREEIS GVKLESIGIY QILSIYSTVA 540 SSLALAIMVA GLSLWMCSNG SLQCRICI 568
3-38	**Sequences**	
3-38-1	Sequence Number [ID]	38
3-38-2	Molecule Type	DNA
3-38-3	Length	1466
3-38-4	Features	**misc_feature 1..1466**
	Location/Qualifiers	note=Influenza H1N1&H5N1 NA consensus Sequence **source 1..1466** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-38-5	Residues	ggtaccgaat tcgccaccat ggactggacc tggatcctgt tcctggtggc cgctgccacc 60 cgggtgcaca gcatgaaccc caaccagaag atcatcacca tcggcagcat ctgcatggtg 120 atcggcatcg tgagcctgat gctgcagatc ggcaacatga tcagcatctg ggtgtcccac 180 agcatccaga ccggcaacca gcaccaggcc gagcccatca gcaacaccaa ctttctgacc 240 gagaaggccg tggccagcgt gaccctggcc ggcaacagca gcctgtgccc catcagcggc 300 tgggccgtgt acagcaagga caacagcatc cggatcggca gcaagggcga cgtgttcgtg 360 atccgggagc ccttcatcag ctgcagccac ctggaatgcc ggaccttctt cctgacccag 420 ggggccctgc tgaacgacaa gcacagcaac ggcaccgtga aggacagaag ccctaccgg 480 accctgatga gctgccccgt gggcgaggcc cccagcccct acaacagccg gttcgagagc 540 gtggcctggt ccgccagcgc ctgccacgac ggcaccagct ggctgaccat cggcatcagc 600 ggccctgaca acggcgccgt ggccgtgctg aagtacaacg gcatcatcac cgacaccatc 660 aagagctggc ggaacaacat cctgcggacc caggaaagcg agtgcgcctg cgtgaacggc 720 agctgcttca ccgtgatgac cgacggcccc agcaacggcc aggccagcta caagatcttc 780 aagatggaaa agggcaaggt ggtgaagagc gtggagctgg acgcccccaa ctaccactac 840 gaggaatgca gctgctaccc cgacgccggc gagatcacct gcgtgtgccg ggacaactgg 900 cacggcagca accggccctg ggtgtccttc aaccagaacc tggaatacca gatcggctac 960 atctgcagcg gcgtgttcgg cgacaacccc aggcccaacg atggcaccgg cagctgcggc 1020 cctgtgagcg ccaacggcgc ctacggcgtg aagggcttca gcttcaagta cggcaacggc 1080 gtgtggatcg gccggaccaa gagcaccaac agcagatccg gcttcgagat gatctgggac 1140 cccaacggct ggaccgagac cgacagcagc ttcagcgtga agcaggacat cgtggccatc 1200 accgactggt ccggctacag cggcagcttc gtgcagcacc ccgagctgac cggcctggac 1260 tgcatccggc cctgcttttg ggtggagctg atcagaggca ggcccaaaga gagcaccatc 1320 tggaccagcg gcagcagcat cagcttttgc ggcgtgaaca gcgacaccgt gagctggtcc 1380 tggcccgacg gcgccgagct gcccttcacc atcgacaagt acccctacga cgtgcccgac 1440 tacgcctgat gagcggccgc gagctc 1466
3-39	**Sequences**	
3-39-1	Sequence Number [ID]	39
3-39-2	Molecule Type	AA
3-39-3	Length	476
3-39-4	Features	**REGION 1..476**
	Location/Qualifiers	note=Influenza H1N1&H5N1 NA consensus sequence **source 1..476** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	

3-39-5	Residues	MDWTWILFLV AAATRVHSMN PNQKIITIGS ICMVIGIVSL MLQIGNMISI WVSHSIQTGN 60 QHQAEPISNT NFLTEKAVAS VTLAGNSSLC PISGWAVYSK DNSIRIGSKG DVFVIREPFI 120 SCSHLECRTF FLTQGALLND KHSNGTVKDR SPYRTLMSCP VGEAPSPYNS RFESVAWSAS 180 ACHDGTSWLT IGISGPDNGA VAVLKYNGII TDTIKSWRNN ILRTQESECA CVNGSCFTVM 240 TDGPSNGQAS YKIFKMEKGK VVKSVELDAP NYHYEECSCY PDAGEITCVC RDNWHGSNRP 300 WVSFNQNLEY QIGYICSGVF GDNPRPNDGT GSCGPVSANG AYGVKGFSFK YGNGVWIGRT 360 KSTNSRSGFE MIWDPNGWTE TDSSFSVKQD IVAITDWSGY SGSFVQHPEL TGLDCIRPCF 420 WVELIRGRPK ESTIWTSGSS ISFCGVNSDT VSWSWPDGAE LPFTIDKYPY DVPDYA 476
3-40	**Sequences**	
3-40-1	Sequence Number [ID]	40
3-40-2	Molecule Type	DNA
3-40-3	Length	875
3-40-4	Features	**misc_feature 1..875**
	Location/Qualifiers	note=Influenza H1N1&H5N1 M1 consensus sequence **source 1..875** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-40-5	Residues	ggtaccggat ccgccaccat ggactggacc tggattctgt tcctggtggc cgctgccacc 60 cgggtgcaca gcatgagcct gctgaccgag gtggagacct acgtgctgtc catcatcccc 120 agcggccctc tgaaggccga gatcgcccag cggctggaag atgtgttcgc cggcaagaac 180 accgacctgg aagccctgat ggaatggctg aaaacccggc ccatcctgag ccccctgacc 240 aagggcatcc tgggcttcgt gttcaccctg accgtgccca gcgagcgggg cctgcagcgg 300 cggagattcg tgcagaacgc cctgaacggc aacggcgacc ccaacaacat ggaccgggcc 360 gtgaagctgt acaagaagct gaagcgggag atcaccttcc acggcgccaa agaggtggcc 420 ctgagctaca gcacaggcgc cctggccagc tgcatgggcc tgatctacaa ccggatgggc 480 accgtgacca ccgaggtggc cttcggcctg gtgtgcgcca cctgcgagca gatcgccgac 540 agccagcaca gatcccaccg gcagatggcc accaccacca acccctgat ccggcacgag 600 aaccggatgg tcctggcctc caccaccgcc aaggccatgg aacagatggc cggcagcagc 660 gagcaggccg ccgaagccat ggaagtggcc agccaggcca ggcagatggt gcaggccatg 720 cggaccatcg gcacccaccc cagcagcagc gccggactgc gggacgacct gctggaaaac 780 ctgcaggcct accagaaacg gatgggcgtg cagatgcagc ggttcaagta cccctacgac 840 gtgcccgact acgcctgatg agcggccgcg agctc 875
3-41	**Sequences**	
3-41-1	Sequence Number [ID]	41
3-41-2	Molecule Type	AA
3-41-3	Length	279
3-41-4	Features	**REGION 1..279**
	Location/Qualifiers	note=Influenza H1N1&H5N1 M1 consensus sequence **source 1..279** mol_type=protein organism=synthetic construct
	NonEnglishQualifier Value	
3-41-5	Residues	MDWTWILFLV AAATRVHSMS LLTEVETYVL SIIPSGPLKA EIAQRLEDVF AGKNTDLEAL 60 MEWLKTRPIL SPLTKGILGF VFTLTVPSER GLQRRRFVQN ALNGNGDPNN MDRAVKLYKK 120 LKREITFHGA KEVALSYSTG ALASCMGLIY NRMGTVTTEV AFGLVCATCE QIADSQHRSH 180 RQMATTTNPL IRHENRMVLA STTAKAMEQM AGSSEQAAEA MEVASQARQM VQAMRTIGTH 240 PSSSAGLRDD LLENLQAYQK RMGVQMQRFK YPYDVPDYA 279
3-42	**Sequences**	
3-42-1	Sequence Number [ID]	42
3-42-2	Molecule Type	DNA
3-42-3	Length	1700
3-42-4	Features	**misc_feature 1..1700**
	Location/Qualifiers	note=Influenza H5N1 M2E-NP consensus sequence **source 1..1700** mol_type=other DNA organism=synthetic construct
	NonEnglishQualifier Value	
3-42-5	Residues	ggtaccgaat tcgccaccat ggactggacc tggatcctgt tcctggtcgc tgccgccacc 60 agggtgcaca gcagcctgct gaccgaggtg gagaccccca cccggaacga gtggggctgc 120 cggtgcagcg acagcagcga ccggggcagg aagcggagaa gcgccagcca gggcaccaag 180 cggagctacg agcagatgga aacaggcggc gagcggcaga acgccaccga gatccgggcc 240 agcgtgggca gaatggtcgg cggcatcggc cggttctaca tccagatgtg caccgagctg 300 aagctgtccg actacgaggg ccggctgatc cagaacagca tcaccatcga gcggatggtg 360 ctgtccgcct tcgacgagcg gcggaacaga tacctggaag agcaccccag cgccggcaag 420 gaccccaaga aaaccggcgg acccatctac cggcggaggg acggcaagtg ggtgcgggag 480 ctgatcctgt acgacaaaga ggaaatccgg cggatctggc ggcaggccaa caacggcgag 540 gacgccacag ccggcctgac ccacctgatg atctggcaca gcaacctgaa cgacgccacc 600 taccagcgga caagggctct ggtccggacc ggcatggacc cccggatgtg cagcctgatg 660 cagggcagca cactgcccag aagaagcgga gccgctggcg cagccgtgaa gggcgtgggc 720 accatggtga tggaactgat ccggatgatc aagcggggca tcaacgaccg gaatttttgg 780 aggggcgaga acggcaggcg gacccggatc gcctacgagc ggatgtgcaa catcctgaag 840

		ggcaagttcc agacagccgc ccagcgggcc atgatggacc aggtccggga gagccggaac 900 cccggcaacg ccgagatcga ggacctgatc ttcctggcca gaagcgccct gatcctgcgg 960 ggcagcgtgg cccacaagag ctgcctgccc gcctgcgtgt acggactggc cgtggccagc 1020 ggctacgact tcgagcggga gggctacagc ctggtcggca tcgacccctt ccggctgctg 1080 cagaactccc aggtgttcag cctgatccgg cccaacgaga accccgccca caagtcccag 1140 ctggtctgga tggcctgcca cagcgccgcc ttcgaggatc tgagagtgag cagcttcatc 1200 cggggcacca gagtggtgcc caggggccag ctgtccacca ggggcgtgca gatcgccagc 1260 aacgagaaca tggaagccat ggacagcaac accctggaac tgcggagccg gtactgggcc 1320 atccggacca gaagcggcgg caacaccaac cagcagcggg ccagcgccgg acagatcagc 1380 gtgcagccca ccttctccgt gcagcggaac ctgcccttcg agagggccac catcatggcc 1440 gccttcaccg gcaacaccga gggccggacc agcgacatgc ggaccgagat catcaggatg 1500 atggaaagcg ccaggcccga ggacgtgagc ttccagggca ggggcgtgtt cgagctgtcc 1560 gatgagaagg ccaccaaccc catcgtgccc agcttcgaca tgaacaacga gggcagctac 1620 ttcttcggcg acaacgccga ggaatacgac aactacccct acgacgtgcc cgactacgcc 1680 tgatgagcgg ccgcgagctc 1700
3-43	**Sequences**	
3-43-1	Sequence Number [ID]	43
3-43-2	Molecule Type	AA
3-43-3	Length	554
3-43-4	Features	**REGION 1..554**
	Location/Qualifiers	note=Influenza H5N1 M2E-NP consensus sequence
		source 1..554
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-43-5	Residues	MDWTWILFLV AAATRVHSSL LTEVETPTRN EWGCRCSDSS DRGRKRRSAS QGTKRSYEQM 60 ETGGERQNAT EIRASVGRMV GGIGRFYIQM CTELKLSDYE GRLIQNSITI ERMVLSAFDE 120 RRNRYLEEHP SAGKDPKKTG GPIYRRRDGK WVRELILYDK EEIRRIWRQA NNGEDATAGL 180 THLMIWHSNL NDATYQRTRA LVRTGMDPRM CSLMQGSTLP RRSGAAGAAV KGVGTMVMEL 240 IRMIKRGIND RNFWRGENGR RTRIAYERMC NILKGKFQTA AQRAMMDQVR ESRNPGNAEI 300 EDLIFLARSA LILRGSVAHK SCLPACVYGL AVASGYDFER EGYSLVGIDP FRLLQNSQVF 360 SLIRPNENPA HKSQLVWMAC HSAAFEDLRV SSFIRGTRVV PRGQLSTRGV QIASNENMEA 420 MDSNTLELRS RYWAIRTRSG GNTNQQRASA GQISVQPTFS VQRNLPFERA TIMAAFTGNT 480 EGRTSDMRTE IIRMMESARP EDVSFQGRGV FELSDEKATN PIVPSFDMNN EGSYFFGDNA 540 EEYDNYPYDV PDYA 554