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(54) **MATERIAU GENETIQUE SOUS FORME DE CASSETTES D'IMMUNISATION A BASE D'ADN DE VIF ATTENUE POUR DES VACCINS GENETIQUES**

(54) **ATTENUATED VIF DNA IMMUNIZATION CASSETTES FOR GENETIC VACCINES**

(57) L'invention concerne des molécules d'acide nucléique codant des protéines du facteur d'infectiosité de virion vif non fonctionnel atténué. On insère ces molécules d'acide nucléique dans des vecteurs d'expression de recombinaison et on les administre à des mammifères, de manière à provoquer une réponse immune cellulaire et humorale à ce produit de protéines codées.

(57) The present invention is directed to nucleic acid molecules encoding attenuated, non-functional virion infectivity factor (vif) proteins. The nucleic acid molecules of the invention are inserted into recombinant expression vectors and administered to mammals in order to induce a cellular and humoral immune response to the encoded protein product.

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(54) **Title:** ATTENUATED VIF DNA IMMUNIZATION CASSETTES FOR GENETIC VACCINES

(57) **Abstract**

The present invention is directed to nucleic acid molecules encoding attenuated, non–functional virion infectivity factor (*vif*) proteins. The nucleic acid molecules of the invention are inserted into recombinant expression vectors and administered to mammals in order to induce a cellular and humoral immune response to the encoded protein product.

ATTENUATED VIF DNA IMMUNIZATION CASSETTES FOR GENETIC VACCINES

ACKNOWLEDGMENT OF GOVERNMENT RIGHTS

This invention was made with Government support from the National Institutes of Health. The Government has certain rights in this invention.

FIELD OF THE INVENTION

The invention relates to the preparation and use of attenuated, nonfunctional HIV *vif* immunization cassettes as genetic vaccines for pathogenic genes.

BACKGROUND OF THE INVENTION

Vaccination and immunization generally refer to the introduction of a non-virulent agent against which an individual's immune system can initiate an immune response which will then be available to defend against challenge by a pathogen. The immune system identifies invading "foreign" compositions and agents primarily by identifying proteins and other large molecules which are not normally present in the individual. The foreign protein represents a target against which the immune response is made.

The immune system can provide multiple means for eliminating targets that are identified as foreign. These means include humoral and cellular responses which participate in antigen recognition and elimination. Briefly, the humoral response involves B cells which produce antibodies that specifically bind to antigens. There are two arms of the cellular immune response. The first involves helper T cells which produce cytokines and

elicit participation of additional immune cells in the immune response. The second involves killer T cells, also known as cytotoxic T lymphocytes (CTLs), which are cells capable of recognizing antigens and attacking the antigen including the cell or particle it is attached to.

Vaccination has been singularly responsible for conferring immune protection against several human pathogens. In the search for safe and effective vaccines for immunizing individuals against infective pathogenic agents such as viruses, bacteria, and infective eukaryotic organisms, several strategies have been employed thus far. Each strategy aims to achieve the goal of protecting the individual against pathogen infection by administering to the individual, a target protein associated with the pathogen which can elicit an immune response. Thus, when the individual is challenged by an infective pathogen, the individual's immune system can recognize the protein and mount an effective defense against infection. There are several vaccine strategies for presenting pathogen proteins which include presenting the protein as part of a non-infective or less infective agent or as a discreet protein composition.

One strategy for immunizing against infection uses killed or inactivated vaccines to present pathogen proteins to an individual's immune system. In such vaccines, the pathogen is either killed or otherwise inactivated using means such as, for example, heat or chemicals. The administration of killed or inactivated pathogen into an individual presents the pathogen to the individual's immune system in a noninfective form and the individual can thereby mount an immune response against it. Killed or inactivated pathogen vaccines provide protection by directly generating T-helper and humoral immune responses against the pathogenic immunogens. Because the pathogen is killed or otherwise inactivated, there is little threat of infection.

Another method of vaccinating against pathogens is to provide an attenuated vaccine. Attenuated vaccines are essentially live vaccines which exhibit a reduced infectivity. Attenuated vaccines are often produced by passaging several generations of the pathogen through a permissive host until the progeny agents are no longer virulent. By using an attenuated vaccine, an agent that displays limited infectivity may be employed to elicit an immune response against the pathogen. By maintaining a certain level of infectivity, the attenuated vaccine produces a low level infection and elicits a stronger immune response than killed or inactivated vaccines. For example, live attenuated vaccines, such as the poliovirus

and smallpox vaccines, stimulate protective T-helper, T-cytotoxic, and humoral immunities during their nonpathogenic infection of the host.

Another means of immunizing against pathogens is provided by recombinant vaccines. There are two types of recombinant vaccines: one is a pathogen in which specific genes are deleted in order to render the resulting agent non-virulent. Essentially, this type of recombinant vaccine is attenuated by design and requires the administration of an active, non-virulent infective agent which, upon establishing itself in a host, produces or causes to be produced antigens used to elicit the immune response. The second type of recombinant vaccine employs infective non-virulent vectors into which genetic material that encode target antigens is inserted. This type of recombinant vaccine similarly requires the administration of an active infective non-virulent agent which, upon establishing itself in a host, produces or causes to be produced, the antigen used to elicit the immune response. Such vaccines essentially employ infective non-virulent agents to present pathogen antigens that can then serve as targets for an anti-pathogen immune response. For example, the development of vaccinia as an expression system for vaccination has theoretically simplified the safety and development of infectious vaccination strategies with broader T-cell immune responses.

Another method of immunizing against infection uses subunit vaccines. Subunit vaccines generally consist of one or more isolated proteins derived from the pathogen. These proteins act as target antigens against which an immune response may be mounted by an individual. The proteins selected for subunit vaccine are displayed by the pathogen so that upon infection of an individual by the pathogen, the individuals immune system recognizes the pathogen and mounts a defense against it. Because subunit vaccines are not whole infective agents, they are incapable of becoming infective. Thus, they present no risk of undesirable virulent infectivity that is associated with other types of vaccines. It has been reported that recombinant subunit vaccines such as the hepatitis B surface antigen vaccine (HBsAg) stimulate a more specific protective T-helper and humoral immune response against a single antigen. However, the use of this technology to stimulate board protection against diverse pathogens remains to be confirmed.

The construction of effective vaccines is complicated by several factors which include the pathobiology of the pathogen and the specificities of the of the host immune response. Recently a novel tool for understanding the immune component in these

interactions has become available in the form of genetic immunization or DNA vaccination. Tang, *et al.*, *Nature*, **1992**, *356*, 152; Fynan, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 11478; Ulmer, *et al.*, *Science*, **1993**, *259*, 1745; and Wang, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 4156. The ability of this approach was demonstrated to produce broad immune responses against structural and enzymatic gene products of HIV-1 and outlined a strategy for development of a possible prophylactic vaccine for HIV-1. This strategy utilized multiple gene expression cassettes encoding *gag/pol/rev* as well as *env/rev* and accessory gene immunogens. Studies clearly demonstrated that rodents and primates can be successfully immunized with HIV-1 structural and envelope genes. Wang, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 4156 and Wang, *et al.*, *DNA Cell Biol.*, **1993**, *12*, 799. A genetic strategy for construction of immunogen expression cassettes from a pathogenic gene which can be broadly applied in order to use DNA immunogens against a variety of pathogens is needed.

Primate lentiviral genomes contain genes encoding novel regulatory and accessory proteins as well as proteins with structural and enzymatic functions. The regulatory genes, *tat* and *rev*, and the accessory genes, *nef*, *vif*, *vpr*, *vpu*, and *vpx*, are well conserved in many lentiviruses, including HIV and SIV. The well conserved nature of these genes implies that their protein products play a critical role in viral pathogenesis *in vivo*. Initial *in vitro* experiments seemed to demonstrate that *tat* and *rev* were essential for viral replication, while the accessory genes were considered nonessential. Cullen, *et al.*, *Cell*, **1989**, *58*, 423 and Desrosiers, *AIDS Res. Human Retroviruses*, **1992**, *8*, 411. Further analyses, however, has revealed that defects within the accessory gene result in severe impairment or delay in viral replication *in vitro* (Gabudza, *et al.*, *J. Virol.*, **1992**, *66*, 6489 and Gibbs, *et al.*, *AIDS Res. Human Retroviruses*, **1994**, *10*, 343) and *in vivo* (Aldrovandi, *et al.*, *J. Virol.*, **1996**, *70*, 1505). Native defective accessory genes have been reported *in vivo* and may be an end product of an effective host immune response. The accessory genes are, therefore, presently considered to be determinants of virus virulence. Trono, *Cell*, **1995**, *82*, 189. They contain few "hot spots" and may be less susceptible to mutations leading to the production of "escape" virus variants, emphasizing their importance in the viral life cycle. In addition, the protein products of these genes are immunogenic *in vivo*. As a group, they represent twenty percent of the possible anti-viral immune targets. Ameisen, *et al.*, *Int. Conf. AIDS*, **1989**, *5*, 533 and Lamhamedi-Cherradi, *et al.*, *AIDS*, **1992**, *6*, 1249. Their immunogenicity and low functional

mutagenicity combine to make the accessory genes attractive elements in the design of future anti-viral immune therapeutics. The production of accessory gene immunogens poses specific immunologic and pathogenic complications for a viral vaccine design, however, due to the role of the accessory gene protein products as determinants of viral virulence. A potential accessory gene-based genetic vaccine would need to be accessible to the host's immune response against native viral accessory gene products without enhancing viral replication. Accordingly, a major goal is to design a safe and effective genetic anti-HIV vaccine, which includes the *vif* (virion infectivity factor) accessory gene as part of a multi-component genetic immunogen.

The *vif* gene encodes a 23 kDa late viral protein (vif) from a singly spliced, rev-dependent 5 kb transcript. Arya, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 2209; Garrett, *et al.*, *J. Virol.*, **1991**, *65*, 1653; Schwartz, *et al.*, *Virol.*, **1991**, *183*, 677; and Sodroski, *et al.*, *Science*, **1986**, *231*, 1549. *Vif* is highly conserved among HIV-1 isolates and is present in other lentiviruses, such as Feline Immunodeficiency Virus (FIV), Bovine Immunodeficiency Virus (BIV), Visna virus, HIV-2, and SIV. Myers, *et al.*, *Human Retrovir. AIDS*, **1991** and Shackett, *et al.*, *Virol.*, **1994**, *204*, 860. Earlier analyses of *in vivo vif* genetic variation have shown that most *vif* sequences are intact reading frames and the presence of intact *vif* does not have a correlation with disease status. Sova, *et al.*, *J. Virol.*, **1995**, *69*, 2557 and Wieland, *et al.*, *Virol.*, **1994**, *203*, 43. However, sequential analyses of a region containing *vif*, *vpr*, *vpu*, *tat*, and *rev* genes from a HIV-1 infected long-term progressor revealed the presence of inactivating mutations in 64% of the clones. Michael, *et al.*, *J. Virol.*, **1995**, *69*, 4228. HIV-1 infected subjects have been shown to carry antibodies which recognize recombinant vif protein (Kan, *et al.*, *Science*, **1986**, *231*, 1553; Schwander, *et al.*, *J. Med. Virol.*, **1992**, *36*, 142; and Wieland, *et al.*, *AIDS Res. Human Retrovir.*, **1991**, *7*, 861) suggesting that the protein is expressed and is immunogenic during natural infection (Volsky, *et al.*, *Curr. Topics Micro. Immunol.*, **1995**, *193*, 157.

Due to vif's ability to activate viral replication in *trans*, an attenuated genetic vaccine design, similar to those utilized in the production of vaccines derived from toxic viral, bacterial, or parasitic components was employed in the present invention. The sequence variation and immunogenic potential present in *vif* genes derived from HIV-1 infected subjects was analyzed. Prototypic genetic variants were selected and the ability of those

clones to induce humoral and cellular immune responses was studied in animals. The selected *vif* genetic variants were also functionally characterized through transcomplementation assays utilizing cells infected with a vif-defective HIV-1 clone. Attenuated, nonfunctional *vif* clones are demonstrated to induce immune responses capable of destroying native pathogen.

SUMMARY OF THE INVENTION

The present invention relates to a purified attenuated, non-functional *vif* protein.

The present invention relates to a *vif* protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23.

The present invention relates to an isolated nucleic acid molecule comprising a nucleotide sequence encoding an attenuated, non-functional *vif* protein.

The present invention relates to a nucleic acid molecule encoding a *vif* protein which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

The present invention relates to a nucleic acid molecule encoding a *vif* protein which comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46.

The present invention relates to a pharmaceutical composition comprising the nucleic acid molecule encoding an attenuated, non-functional *vif* protein in a pharmaceutically acceptable carrier or diluent.

The present invention relates to a recombinant expression vector comprising a nucleic acid molecule comprising a nucleotide sequence encoding an attenuated, non-functional *vif* protein.

The present invention relates to a host cell comprising a recombinant expression vector comprising a nucleic acid molecule encoding an attenuated, non-functional *vif* protein

The present invention relates to a purified antibody directed against an attenuated, non-functional *vif* protein.

The present invention relates to a method of immunizing a mammal against a virus comprising administering to cells of said mammal, a nucleic acid molecule that comprises a nucleotide sequence that encodes an attenuated, non-functional *vif* protein, wherein said nucleic acid molecule is expressed in said cells.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a comparison of the deduced amino acid sequences of *vif* derived from transmitter and non-transmitter mothers with well characterized HIV-1 molecular clones PNL43, SF-2, and Zr6. T-#, clones from transmitter subject; N-#, clones from non-transmitter subject; --, identity with the consensus sequence (Con; SEQ ID NO:1); .., represents gap; *, a stop codon.

Figure 2 shows a 10% SDS-PAGE of immunoprecipitates. Expression of HIV-1 *vif* clones derived from transmitter and non-transmitter mothers. *Vif* expression plasmids were used for coupled *in vitro* transcription/translation according to the manufacturer's instructions (Promega). Immunoprecipitation of the *in vitro* translated proteins was performed with *vif* antiserum as described herein. The designation of the *vif* clones is indicated on the top. The clone numbers designated with T-** and N-** are derived from the transmitter and non-transmitter mothers respectively. pCVif is the *vif* expression plasmid of HIV-1_{SF2}.

Figures 3A and 3B show the results of an enzyme linked immunoabsorbent assay (ELISA) of anti-vif antibody responses in mice after immunization with a DNA construct expressing *vif*. Mouse sera was diluted in blocking buffer at a dilution of 1:500 and

assayed as described herein. In Figure 3A, mice were immunized with 50 μg of DNA. In Figure 3B, mice were immunized with 100 μg of DNA per injection.

Figure 4 shows the results of a chromium release assay whereby lysis of murine targets (p815) expressing *vif* protein by splenocytes from mice immunizied with *vif* expression constructs. p815 cells (1 x 10^5/ml) were infected with vaccinia expressing *vif* (VV:gag) and incubated for 16 hours to express the Vif protein. The target cells were labeled with ^{51}Cr for 1-2 hours and used to incubate the stimulated splenocytes for 6 hours. Specific lysis (%) was calculated according to the formula described herein.

Figures 5A, 5B, 5C and 5D show the results of a chromium release assay whereby lysis of HeLa CD4+/D^d cells infected with clinical HIV-1 isolate by splenocytes from mice immunized with *vif* expression cassette. HeLa CD4+/D^d cells (10^6) were infected with cell-free HIV-1 clinical isolate followed by a week incubation to allow the cells to infect and express viral proteins. One week postinfection, the target cells were labeled with ^{51}Cr for 1-2 hours and used to incubate the stimulated splenocytes for 6 hours. Specific lysis (%) was calculated according to the formula described herein.

Figure 6 shows the results of a proliferation assay showing activation and T cell proliferative response to recombinant Vif. Recombinant Vif (10 μg/ml) was plated in each well to stimulate proliferation of T cells. Lectin PHA (10 μg/ml) was used as a polyclonal stimulator positive control. Stimulation index was calculated as the level of radioactivity detected from the cells stimulated with specific protein divided by the level detected from the cells in media. Lanes 1a and 1b are from mice immunized with 50 and 100 μg of pCVif; Lanes 2a and 2b are from mice immunized with 50 and 100 μg of clone T-35; Lanes 2a and 2b are from mice immunized with 50 and 100 μg of clone N-15.

Figures 7A-7F show the amino acid sequences of preferred attenuated, non-functional *vif* proteins of the present invention.

Figures 8A-8E show the nucleotide sequences of preferred attenuated, non-functional *vif* proteins of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

One of the major goals of AIDS research is the development of a vaccine against the HIV-1 virus. An effective vaccine should elicit strong humoral response along

with an efficient and broad CTL response. This task is complicated because of the genetic heterogeneity of the HIV-1 virus. HIV-1 reverse transcriptase (RT) is prone to error and lacks the ability to proof-read, resulting in a mutation rate of 10^{-4} per cycle per genome. Dougherty, *et al.*, *J. Virol.*, **62**, 2817. HIV-1 genome sequence variation has been observed in viruses isolated from different individuals as well as in virus isolated from a single person at different time points. Fisher, *et al.*, *Nature*, **1988**, *334*, 444 and Meyerhans, *et al.*, *Cell*, **1989**, *58*, 901. Based upon a large number of sequence analysis data, it is apparent that the structural genes *env*, *gag* and *pol* are the major target for mutations which lead to escape-variant viruses in patients by changing the neutralizing antibody and/or CTL epitopes. Pircher, *et al.*, *Nature*, **1990**, *346*, 629; Reitz, *et al.*, *Cell*, **1988**, *54*, 57; and Wolfs, *et al.*, *Virol.*, **1991**, *185*, 195. Despite this, earlier experiments have indicated that structural and enzymatic genes of HIV-1 can be used successfully as nucleic acid-based vaccines in different animal models (Wang, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 4156; and Wang, *et al.*, *AIDS*, **1995**, *9 (Suppl A)*, S159) and prophylactic as well as therapeutic studies for DNA vaccines have commenced. The present invention is directed to development of *vif*, a HIV-1 accessory gene, as an immunogen cassette. When used in concert with other HIV-1 genes a broad immune response against all viral components may be induced, thus mimicking many aspects of the immune responses induced by a live attenuated vaccine.

In the present invention, induction of vif-specific humoral and cellular immune responses in mice have been observed to directly correlate with the concentration of DNA injected and number of boosts. Similar results were observed in T-cell proliferation and CTL assays, demonstrating that vif genes are immunogenic *in vivo*. *Vif* is known to present in both soluble and membrane associated form. Goncalves, *et al.*, *J. Virol.*, **1994**, *68*, 704. Although anti-vif antibodies and vif-specific CTL responses have been shown in HIV-1 positive patients, epitopes involved in the presentation of vif to the immune system have not yet been defined. Lamhamedi-Cherradi, *et al.*, *AIDS*, **1992**, *6*, 1249. How vif becomes exposed to the humoral immune system is unclear in these studies. The observed different immune response in the clones of the present invention suggest that the mutations in T-35, N-15 and pCVif may be associated with changes in antibody/CTL responses.

It is significant to note that some the point mutations present in all the T or N derived clones indicate that these mutations may be responsible for the difference in

complementation and/or immune responses observed. Further mutational analysis of vif help resolve answer the regions involved in complementation. Proposed sites of vif activity include viral DNA synthesis, gpl20 synthesis and transport, and gag processing. Borman, *et al.*, *J. Virol.*, **1995**, *69*, 2058; Sakai, *et al.*, *J. Virol.*, **1993**, *67*, 1663; and Von Schwedler, *et al.*, *J. Virol.*, **1993**, *67*, 4945. Transcomplementation experiments with vif-defective HIV-1 provirus and wild-type HIV-1 vif-expressing cell lines indicate that vif acts at a late stage in virus replication/maturation and that *vif* transcomplementation occurs across HIV-1 strains. Blanc, *et al.*, *Virol.*, **1993**, *193*, 186 and Hevey, *et al.*, *Virus Res.*, **1994**, *33*, 269. Earlier experiments have shown that sera from the nontransmitter subject (N1) contains a high antibody titer against envelope protein and nonreplicating virus; whereas sera from the transmitter patient (TI) contains very low antibody titers against envelop proteins and highly replicating virus. Velpandi, *et al.*, *DNA Cell Biol.*, **1996**, *15*, 571. These results correlate with the trans-complementation results observed in the present invention.

The present invention relates to isolated nucleic acid molecules comprising a nucleotide sequence encoding an attenuated, non-functional *vif* protein. As used herein, the term "attenuated, non-functional *vif* protein" is meant to refer to *vif* proteins that have no or reduced virion infectivity function compared to wild-type *vif*. In some embodiments of the invention, the nucleic acid molecules encode an attenuated, non-functional *vif* protein wherein the nucleotide sequence comprises deletions, additions, a point mutation(s), multiple substitutions, or introduction of a stop codon to render a shortened protein. In preferred embodiments of the invention, the isolated nucleic acid molecules of the invention encode a *vif* protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23. In other preferred embodiments of the invention, the isolated nucleic acid molecules encode a *vif* protein and comprise a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46.

The nucleic acid molecules of the invention may be obtained from patients infected with the human immunodeficiency virus as described below in the Examples. Alternatively, the nucleic acid molecules of the invention may be prepared using the wild-type *vif* nucleotide sequence. The vif expression plasmid, pCVif, contains the *vif* gene from the well-characterized HIV-1 molecular clone, pHXB2, under the control of the cytomegalovirus (CMV) immediate early promoter, within the backbone plasmid, pRc/CMV (Invitrogen, San Diego, CA) as described in Nagashunmugam, *et al.*, *DNA Cell Biol.*, **1996**, *15*, 353, incorporated herein by reference. This nucleic acid molecule may be used to prepare additional nucleic acid molecules encoding attenuated, non-functional *vif* proteins.

A number of methods can be used to design specific mutations in wild-type nucleic acid molecules to produce nucleic acid molecules encoding attenuated, non-functional *vif* proteins. For example, oligonucleotide-mediated mutagenesis is commonly used to add, delete, or substitute nucleotides in a segment of DNA whose sequence is known. Such methods are taught in, for example, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989), pages 15.51 to 15.73, which is incorporated herein by reference. Briefly, the protocol for oligonucleotide-mediated mutagenesis involves the following steps: 1) cloning of an appropriate fragment of DNA, such as the *vif* nucleotide sequence from the pCVif expression plasmid, into a bacteriophage M13 vector; 2) preparation of single-stranded DNA from the recombinant bacteriophage M13; 3) design and synthesis of mutagenic oligonucleotides; 4) hybridization of the mutagenic oligonucleotides to the target DNA; 5) extension of the hybridized oligonucleotide by DNA polymerase; 6) transfection of susceptible bacteria; 7) screening of bacteriophage plaques for those carrying the desired mutation; 8) preparation of single-stranded DNA from the mutagenized recombinant bacteriophage; 9) confirmation by sequencing that the mutagenized bacteriophage M13 DNA carries the desired mutation and no other mutation; 10) recovery of the mutated fragment of DNA from the double-stranded replicative form of the recombinant bacteriophage M13; and 11) substitution of the mutagenized fragment for the corresponding segment of wild-type DNA in the desired expression vector.

Design and synthesis of the mutagenic oligonucleotides, which are tailored to the desired mutation in the nucleic acid molecule encoding *vif*, is described in detail in, for example, Sambrook et al., *Molecular Cloning a Laboratory Manual*, Second Ed. Cold Spring Harbor Press (1989), pages 15.54 to 15.56, which is incorporated herein by reference. For example, to substitute, add, or delete a single nucleotide into the wild-type *vif* nucleotide sequence, oligonucleotides of about 17-19 nucleotides in length which carry the mismatched nucleotide at the center or at one of the two nucleotide positions immediately 3' of the center are prepared. To substitute, add, or delete two or more contiguous nucleotides into the wild-type *vif* nucleotide sequence, oligonucleotides of about 25 or more nucleotides in length are prepared. These oligonucleotides comprise about 12 to 15 perfectly matched nucleotides on either side of the central looped-out region which contains the added or substituted nucleotides, or represents the portion of the wild-type DNA that is looped out. Using the strategy described above, one skilled in the art can prepare nucleic acid molecules having deletions, additions, substitutions, or premature stop codons, which encode attenuated, non-functional *vif* proteins. Oligonucleotide-mediated mutagenesis procedures are widely known to those skilled in the art.

Alternately, the nucleic acid molecules of the invention can be prepared using DNA synthesizers by standard DNA methodology. One skilled in the art readily understands that the genetic code is degenerate and, therefore, could prepare numerous DNA sequences encoding the same protein. In addition, one skilled in the art readily understands that amino acids can be substituted by other amino acids such that conservative substitutions are made. Accordingly, one skilled in the art can prepare nucleic acid molecules of the invention encoding attenuated, non-functional *vif* proteins.

Preferred nucleic acid molecules of the invention encode attenuated, non-functional *vif* proteins having the amino acid (a.a.) and nucleotide sequences (nt.) (represented by particular SEQ ID Numbers) in Table 1. The specific amino acid sequences are shown in Figure 1 and Figures 7A-7F. The specific nucleotide sequences are shown in Figures 8A-8E.

Table 1

Vif Protein	SEQ ID NO:		Vif Protein	SEQ ID NO:	
	a.a.	nt.		a.a.	nt.
N13	4	27	T3	14	37
N15	5	28	T4	15	38
N17	6	29	T35	16	39
N22	7	30	T37	17	40
N23	8	31	T38	18	41
N24	9	32	T39	19	42
N26	10	33	T40	20	43
N27	11	34	T42	21	44
N29	12	35	T43	22	45
N30	13	36	T44	23	46

The present invention also relates to vectors or recombinant expression vectors that comprise a nucleotide sequence that encodes an attenuated, non-functional *vif* protein. As used herein, the term "recombinant expression vector" is meant to refer to a plasmid, phage, viral particle or other vector which, when introduced into an appropriate host, contains the necessary genetic elements to direct expression of the coding sequence that encodes an attenuated, non-functional *vif* protein. In some embodiments of the invention, the vector or recombinant expression vector encodes an attenuated, non-functional *vif* protein wherein the nucleotide sequence comprises deletions, additions, point mutation(s), multiple substitutions, or introduction of a stop codon to render a shortened protein. In preferred embodiments of the invention, the vectors or recombinant expression vectors of the invention encode a *vif* protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23. In other preferred embodiments of the invention, the vectors or recombinant expression vectors of the invention comprise a nucleic

acid molecule encoding a *vif* protein which comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46.

One having ordinary skill in the art can isolate the nucleic acid molecule that encodes an attenuated, non-functional *vif* protein and insert it into an expression vector using standard techniques and readily available starting materials. The coding sequence is operably linked to the necessary regulatory sequences. Expression vectors are well known and readily available. Examples of expression vectors include plasmids, phages, viral vectors and other nucleic acid molecules or nucleic acid molecule containing vehicles useful to transform host cells and facilitate expression of coding sequences. The recombinant expression vectors of the invention are useful for transforming hosts which express an attenuated, non-functional *vif* protein.

The present invention also relates to a host cell that comprises the recombinant expression vector that includes a nucleotide sequence that encodes an attenuated, non-functional *vif* protein. In some embodiments of the invention, the host cell comprises the vector or recombinant expression vector that encodes an attenuated, non-functional *vif* protein wherein the nucleotide sequence comprises deletions, additions, point mutation(s), multiple substitutions, or introduction of a stop codon to render a shortened protein. In preferred embodiments of the invention, the host cells comprises vectors or recombinant expression vectors that encode a *vif* protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23. In other preferred embodiments of the invention, the host cell comprises vectors that comprise a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45

and SEQ ID NO:46. Host cells for use in well known recombinant expression systems for production of proteins are well known and readily available.

The most commonly used prokaryotic system remains *E. coli*, although other systems such as *B. subtilis* and *Pseudomonas* are also useful. Suitable control sequences for prokaryotic systems include both constitutive and inducible promoters including the *lac* promoter, the *trp* promoter, hybrid promoters such as tac promoter, the *lambda* phage Pl promoter. In general, foreign proteins may be produced in these hosts either as fusion or mature proteins. When the desired sequences are produced as mature proteins, the sequence produced may be preceded by a methionine which is not necessarily efficiently removed. Accordingly, the peptides and proteins claimed herein may be preceded by an N-terminal Met when produced in bacteria. Moreover, constructs may be made wherein the coding sequence for the peptide is preceded by an operable signal peptide which results in the secretion of the protein. When produced in prokaryotic hosts in this matter, the signal sequence is removed upon secretion. Examples of prokaryotic host cells include bacteria cells such as *E. coli*, and yeast cells such as *S. cerevisiae*.

A wide variety of eukaryotic hosts are also now available for production of recombinant foreign proteins. As in bacteria, eukaryotic hosts may be transformed with expression systems which produce the desired protein directly, but more commonly signal sequences are provided to effect the secretion of the protein. Eukaryotic systems have the additional advantage that they are able to process introns which may occur in the genomic sequences encoding proteins of higher organisms. Eukaryotic systems also provide a variety of processing mechanisms which result in, for example, glycosylation, carboxy-terminal amidation, oxidation or derivatization of certain amino acid residues, conformational control, and so forth. Commonly used eukaryotic systems include, but are not limited to, yeast, fungal cells, insect cells, mammalian cells, avian cells, and cells of higher plants. In preferred embodiments of the invention insect cells such as *S. frugiperda*, non-human mammalian tissue culture cells chinese hamster ovary (CHO) cells and human tissue culture cells such as HeLa cells are used as host cells. Suitable promoters are available which are compatible and operable for use in each of these host types as well as are termination sequences and enhancers, as e.g. the baculovirus polyhedron promoter. As above, promoters can be either

constitutive or inducible. For example, in mammalian systems, the mouse metallothionene promoter can be induced by the addition of heavy metal ions.

In some embodiments, for example, one having ordinary skill in the art can, using well known techniques, insert DNA molecules into a commercially available expression vector for use in well known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, CA) may be used for production of an attenuated, non-functional *vif* protein in *E. coli*. The commercially available plasmid pYES2 (Invitrogen, San Diego, CA) may, for example, be used for production in *S. cerevisiae* strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, CA) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA I or pcDNA3 (Invitrogen, San Diego, CA) 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 an attenuated, non-functional *vif* 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.

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).

Examples of genetic constructs include the attenuated, non-functional *vif* 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 an attenuated, non-functional *vif* protein from readily available starting materials. Such gene constructs are useful for the production of an attenuated, non-functional *vif* protein.

Nucleic acid molecules that encode an attenuated, non-functional *vif* protein may be delivered to cells using any one of a variety of delivery components, such as recombinant viral expression vectors or other suitable delivery means, so as to affect their introduction and expression in compatible host cells. In general, viral vectors may be DNA viruses such as recombinant adenoviruses and recombinant vaccinia viruses or RNA viruses such as recombinant retroviruses. Other recombinant vectors include recombinant prokaryotes which can infect cells and express recombinant genes. In addition to recombinant vectors, other delivery components are also contemplated such as encapsulation in liposomes, transferrin-mediated transfection and other receptor-mediated means. The invention is intended to include such other forms of expression vectors and other suitable delivery means which serve equivalent functions and which become known in the art subsequently hereto.

In a preferred embodiment of the present invention, DNA is delivered to competent host cells by means of an adenovirus. One skilled in the art would readily understand this technique of delivering DNA to a host cell by such means. Although the invention preferably includes adenovirus, the invention is intended to include any virus which serves equivalent functions.

In another preferred embodiment of the present invention, RNA is delivered to competent host cells by means of a retrovirus. One skilled in the art would readily understand this technique of delivering RNA to a host cell by such means. Any retrovirus which serves to express the protein encoded by the RNA is intended to be included in the present invention.

In another preferred embodiment of the present invention, nucleic acid is delivered through folate receptor means. The nucleic acid sequence to be delivered to a host cell is linked to polylysine and the complex is delivered to the tumor cell by means of the folate receptor. U.S. Patent 5,108,921 issued April 28, 1992 to Low *et al.*, which is incorporated herein by reference, describes such delivery components.

The present invention is also related to purified attenuated, non-functional *vif* proteins. The *vif* proteins of the invention have deletions, additions, point mutation(s), multiple substitutions, or introduction of stop codons to produce peptides that are attenuated and non-functional compared to wild type *vif* protein. In preferred embodiments of the invention, the attenuated, non-functional *vif* proteins of the invention comprise an amino acid

sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23. In other preferred embodiments of the invention, the attenuated, non-functional *vif* proteins of the invention consist of an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23. The *vif* proteins of the invention may be prepared by routine means using readily available starting materials as described above.

The particulars for the construction of expression systems suitable for desired hosts are known to those in the art and are described above. For recombinant production of the protein, the DNA encoding it is suitably ligated into the expression vector of choice and then used to transform the compatible host which is then cultured and maintained under conditions wherein expression of the foreign gene takes place. The proteins of the present invention thus produced are 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 an attenuated, non-functional *vif* protein that is produced using such expression systems. Methods of purifying an attenuated, non-functional *vif* protein from natural sources using antibodies which specifically bind to an attenuated, non-functional *vif* protein may be equally applied to purifying an attenuated, non-functional *vif* protein produced by recombinant DNA methodology.

In addition to producing these proteins by recombinant techniques, automated amino acid synthesizers may also be employed to produce *vpr* protein. It should be further noted that if the proteins herein are made synthetically, substitution by amino acids which are not encoded by the gene may also be made. Alternative residues include, for example, the ω amino acids of the formula $H_2N(CH_2)_nCOOH$ wherein n is 2-6. These are neutral, nonpolar amino acids, as are sarcosine (Sar), t-butylalanine (t-BuAla), t-butylglycine (t-BuGly), N-methyl isoleucine (N-MeIle), and norleucine (Nleu). Phenylglycine, for example, can be

substituted for Trp, Tyr or Phe, an aromatic neutral amino acid; citrulline (Cit) and methionine sulfoxide (MSO) are polar but neutral, cyclohexyl alanine (Cha) is neutral and nonpolar, cysteic acid (Cya) is acidic, and ornithine (Orn) is basic. The conformation conferring properties of the proline residues may be obtained if one or more of these is substituted by hydroxyproline (Hyp).

Pharmaceutical compositions according to the invention comprise a pharmaceutically acceptable carrier in combination with either an attenuated, non-functional *vif* protein or a nucleic acid molecule of the invention encoding the same. In preferred embodiments of the invention, the pharmaceutical composition comprises a recombinant expression vector that encodes a *vif* protein comprising the amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

In other preferred embodiments of the invention, the pharmaceutical composition comprises a nucleic acid molecule encoding a *vif* protein which comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46. Pharmaceutical formulations are well known and pharmaceutical compositions comprising the compounds of the invention may be routinely formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Osol, a standard reference text in this field, which is incorporated herein by reference in its entirety.

The present invention also relates to an injectable pharmaceutical composition that comprises a pharmaceutically acceptable carrier and a compound of the present invention. The compound of the invention is preferably sterile and combined with a sterile pharmaceutical carrier. In some embodiments, for example, the compounds of the invention can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable vehicle. Examples of such vehicles are water, saline,

Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.

An injectable composition may comprise a compound of the invention in a diluting agent such as, for example, sterile water, electrolytes/dextrose, fatty oils of vegetable origin, fatty esters, or polyols, such as propylene glycol and polyethylene glycol. The injectable must be sterile and free of pyrogens.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

The pharmaceutical compositions of the present invention may be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation, or intrathecal or intraventricular administration.

Dosage varies depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art.

According to the invention, the pharmaceutical composition comprising a nucleic acid molecule that encodes a *vif* protein of the invention may be administered directly into the individual or delivered *ex vivo* into removed cells of the individual which are reimplanted after administration. By either route, the genetic material is introduced into cells which are present in the body of the individual. Preferred routes of administration include intramuscular, intraperitoneal, intradermal and subcutaneous injection. Alternatively, the pharmaceutical composition may be introduced by various means into cells that are removed from the individual. Such means include, for example, transfection, electroporation and microprojectile bombardment. After the nucleic acid molecule is taken up by the cells, they are reimplanted into the individual.

The pharmaceutical compositions according to this aspect of the present invention comprise about 0.1 to about 1000 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms of DNA. Most preferably, the pharmaceutical compositions contain about 100 micrograms DNA.

The pharmaceutical compositions according to this aspect of the present invention are formulated according to the mode of administration to be used. One having ordinary skill in the art can readily formulate a nucleic acid molecule that encodes a *vif* protein of the invention. In cases where intramuscular injection is the chosen mode of administration, an isotonic formulation is used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. Isotonic solutions such as phosphate buffered saline may be used. Stabilizers include gelatin and albumin.

DNA-based pharmaceutical agents are being developed as a new generation of vaccines. DNA therapeutics are typically plasmids that contain one or more DNA vaccines are typically plasmids which contain one or more genes from a particular pathogen or undesirable cell. Once injected, the coding sequence of the DNA vaccine is expressed in the patient or vaccinee as protein products and an immune response against the protein product is induced. Examples of protocols for delivering DNA which can be adapted for use with the present invention are described in U.S. Patent No. 5,593,972 issued January 14, 1997 to Weiner, U.S. Patent No. 5,589,466 issued December 14, 1996 to Felgner et al., U.S. Patent

Number 4,945,050 issued July 31, 1990 to Sanford et al., U.S. Patent Number 5,036,006 issued July 30, 1991 to Sanford et al., PCT publication serial number WO 90/11092, PCT publication serial number WO 93/17706, PCT publication serial number WO 93/23552, and PCT publication serial number WO 94/16737 which are each incorporated herein by reference.

In preferred embodiments of the invention, pharmaceutical compositions comprising nucleic acid molecule comprising a nucleotide sequence encoding an attenuated, non-functional *vif* protein is administered to a mammal by the methods described above in order to induce a humoral and/or a cellular immune response to *vif* protein. In other embodiments of the invention, the pharmaceutical compositions of the invention can be co-administered with additional compounds. Such additional compounds include, for example, different viral proteins or nucleic acid molecules encoding a different viral proteins. The different viral proteins include, for example, *gag*, *pol*, *env*, *vpr*, *vpu*, and *tat*, and the like. Such elicited immune responses are protective against HIV or related animal viruses.

The present invention is also directed to antibodies directed against an attenuated, non-functional *vif* protein. As used herein, the term "antibody" is meant to refer to complete, intact antibodies, and Fab fragments and $F(ab)_2$ fragments thereof. Complete, intact antibodies include monoclonal antibodies such as murine monoclonal antibodies, chimeric antibodies and humanized antibodies. In some embodiments, the antibodies specifically bind to an epitope of *vif* or attenuated, non-functional *vif*. Antibodies that bind to an epitope are useful to isolate and purify that protein from both natural sources or recombinant expression systems using well known techniques such as affinity chromatography. Such antibodies are useful to detect the presence of such protein in a sample and to determine if cells are expressing the protein.

Hybridomas which produce antibodies that bind to *vif* protein, and the antibodies themselves, are useful in the isolation and purification of *vif* and attenuated, non-functional *vif* and protein complexes that include *vif* or attenuated, non-functional *vif*. In addition, antibodies may be specific inhibitors of *vif* activity. Antibodies which specifically bind to *vif* or attenuated, non-functional *vif* may be used to purify the protein from natural sources using well known techniques and readily available starting materials. Such antibodies

may also be used to purify the protein from material present when producing the protein by recombinant DNA methodology.

The production of antibodies and the protein structures of complete, intact antibodies, Fab fragments and $F(ab)_2$ fragments and the organization of the genetic sequences that encode such molecules are well known and are described, for example, in Harlow, E. and D. Lane (1988) *ANTIBODIES: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. which is incorporated herein by reference. Briefly, for example, *vif* or attenuated, non-functional *vif*, or an immunogenic fragment thereof, is injected into mice. The spleen of the mouse is removed, the spleen cells are isolated and fused with immortalized mouse cells. The hybrid cells, or hybridomas, are cultured and those cells which secrete antibodies are selected. The antibodies are analyzed and, if found to specifically bind to *vif* or attenuated, non-functional *vif*, the hybridoma which produces them is cultured to produce a continuous supply of antibodies.

The present invention is further illustrated by the following examples, which are not intended to be limiting in any way. All references cited in the present application are incorporated in their entirety.

EXAMPLES

Example 1: Patients

Virus from one HIV-1 positive transmitter mother (T1) and one HIV-1 positive non-transmitter mother (N1) were used in the present invention. Peripheral blood lymphocytes (PBLs) obtained during the subject's third trimester were provided by the Mother Infant Cohort Study, Viral Epidemiology Branch, NCI (Rockville, MD). A follow up examination was performed on the subjects and their offspring in order to determine transmission status.

Example 2: HIV-1 Isolation

Infected primary lymphocytes were co-cultivated with PHA-stimulated normal donor lymphocytes for 2 weeks. Virus production was monitored by: 1) measuring the levels of intracellular HIV-1 reverse transcriptase (RT) (Velpandi, *et al.*, *J. Virol. Meth.*, **1990**, *29*, 291; incorporated herein by reference) and 2) measuring the amount of HIV-1 p24 antigen

released into the medium using a p24 antigen kit (Coulter Corporation), used according to the manufacturer's guidelines.

Example 3: DNA Preparation And PCR Amplification

High molecular weight (genomic) DNA was prepared from the infected PBLs and amplifies through PCR technology as described in Velpandi, *et al.*, *J. Virol. Meth.*, **1990**, *29*, 291, incorporated herein by reference. Briefly, the PCR mixture contained 5 to 10 µg of genomic DNA, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris/HCl (pH 8.0), 800 µM dNTPs, 2.5 units Taq polymerase, 20 pmol oligonucleotide primers and double de-ionized water (ddH_2O) in a final volume of 100 µl. Reaction temperatures and cycling times were: 94°C-denaturing (1 minute), 55°C-annealing (1.5 minutes) and 72°C-extension (2 minutes). The cycle was repeated 35 times. The primer sequences are as follows: Vif(+) 5'-**GAAAGCTT**ATGGAAAACAGATGGCAG-3' (5046-5065) (SEQ ID NO:2); and Vif(-) 5'-**GCAAAGCTT**TCATTGTATGGCTC-3' (5609-5626) (SEQ ID NO:3). The primers were tagged with a HindIII restriction site (in bold) for cloning purposes.

Example 4: Cloning And Sequencing

PCR-amplified product was used for cloning as described in Velpandi, *et al.*, *DNA Cell Biol.*, **1996**, *15*, 571, incorporated herein by reference. Plasmid DNA positive for the *vif* gene was purified by mini preparations (Qiagen, CA) and quantitated by spectrophotometry in preparation for sequencing of the insert. Sequencing reactions were performed using an ABT automated sequencer and Dye Deoxy reactions (Applied Biosystems, Foster City, CA).

Example 5: Sequence Analysis

Sequence alignments were constructed using the Genetics Computer Group Sequence Analysis software package acquired through the Medical School Computer Facility of the University of Pennsylvania VAX system. Homology comparisons of amino acid sequences were carried out by sequence alignment programs.

Example 6: Construction Of Vif-Defective Provirus

HIV-1 proviral DNA, pZr6, was used to construct a vif deletion mutant as described in Nagashunmugam, *et al.*, *DNA Cell Biol.*, **1996**, *15*, 353, incorporated herein by reference. The resulting proviral clone, p911, contains an 80 amino acid deletion in the *vif* gene which does not affect the 3' reading frame. Briefly, HIV-1 proviral DNA pZr6 was derived from primary blood lymphocytes infected with HIV_{Zr6} as described in Srinivasan, *et al.*, *Gene*, **1987**, *52*, 71-82, incorporated herein by reference in its entirety. A deletion was introduced into pZr6 to prepare p911. The mutant was constructed so as not to interfere with the upstream *pol* gene or the downstream *vpr* gene. Plasmid pZr6 contains two *NdeI* sites in the *vif* gene at nucleotide positions 476 and 716. Srinivasan, *et al.*, *Gene*, **1987**, *52*, 71-82. The *NdeI* fragment (477-716) was deleted from pZr6 and the ends were religated to construct p911, an in-frame mutant that has 80 amino acids deleted in the central region of the *vif* protein.

Example 7: Construction Of Vif Expression Vectors

The vif expression plasmid, pCVif, contains the *vif* gene from the well-characterized HIV-1 molecular clone, pHXB2, under the control of the cytomegalovirus (CMV) immediate early promoter, within the backbone plasmid, pRc/CMV (Invitrogen, San Diego, CA) as described in Nagashunmugam, *et al.*, *DNA Cell Biol.*, **1996**, *15*, 353, incorporated herein by reference. The *vif* genes from the maternal samples were cloned into the Invitrogen expression vector, pCDNA3, under the control of the CMV promoter. The *vif* reading frames were verified through sequence analysis using the forward primer, T7, and the reverse primer, SP6. Briefly, to construct a *vif* expression vector (pCVif), an *Eco R1-Eco R1* 1.1 kb fragment from pHxB2 (map coordinates 4,647-5,742; Ratner, *et al.*, *Nature*, **1985**, *313*, 277-284, incorporated herein by reference in its entirety) was cloned under the control of the cytomegalovirus immediate early promoter into plasmid pCDNA3 obtained from Invitrogen. This fragment also contains flanking sequences from parts of the *pol* and *vpr* genes, which are not transcriptionally active as shown in a similar construct by Blanc, *et al.* (*Virology*, **1993**, *193*, 186-192).

Example 8: In Vitro Translation Of Vif

In vitro transcription and translation was performed on 1 µg of *vif* expression construct DNA using T7 RNA polymerase according to the manufacturer's instructions (Promega, Madison, WI). Five (5) µl of the *in vitro* translation reaction products were combined with 500 µl of radioimmunoprecipitation assay buffer and immunopreciptated with rabbit anti-vif antiserum as described. Mahalingam, *et al.*, *Virol.*, **1995**, *214*, 647.

Example 9: Cells

Rhabdomyosarcoma (RD) cells, obtained from the American Type Culture Collection (ATCC), were grown in a monolayer at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% penicillin, 1% streptomycin and 1% L-glutamine. Lymphocytoid cell lines obtained from ATCC were maintained as suspension cultures in RPMI 1640 medium, supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and L-glutamine (540 µg/ml) at 37°C with 5% CO_2. Phytohemagglutinin-stimulated (10 µg/ml) PBLs were maintained in RPMI 1640 medium containing 10% T-cell growth factor.

Example 10: Immunization Of Mice With Vif Constructs

For immunization experiments in mice, 3 different *vif* constructs were used. The *vif* clones selected were T-35 (from transmitter), N-15 (from non-transmitter) and pCVif (*vif* gene of HIV-1_{SF-2}). pCDNA3 vector DNA was used as a negative control. In order to enhance DNA uptake, the quadriceps muscles of BALB/c mice were injected with 100 µl of 0.25% bupivacaine 48 hours before DNA injection. Fifty (50) or 100 µg of each *vif* expression plasmid was injected in a final volume of 100 µl into each of 4 mice. The animals were boosted 3 times at two week intervals.

Example 11: ELISA Binding Of Mouse Serum To rvif Protein

ELISA was performed on mouse serum as described in Wang, *et al.*, *AIDS*, **1995**, *9 (Suppl A)*, S159. Briefly, ELISA plates were coated with recombinant vif (rvif) protein at concentration of 100 ng/well for the binding assays. Mouse sera were diluted (1:100 and 1:500) in blocking buffer, tagged with anti-mouse IgG conjugated to horseradish

peroxidase (HRP) and detected by TMBlue substrate. The non-specific binding and the prebled sera binding were subtracted from the specific binding of the DNA injected animal sera.

Example 12: CTL Assay Using Vaccinia Expressing *vif*

DNA injected mice were sacrified 7 weeks after the first immunization, and their spleens were removed for CTL and T-cell proliferation assays as described in Wang, *et al.*, *DNA Cell Biol.*, **1993**, *12*, 799. Briefly, P815 cells infected with *vif*-expressing vaccinia (VV:gag kindly provided by NIH AIDS Reagent and Reference Program) were used as target cells. Ten (10) μCi of Na_2CrO_4 (^{51}Cr, 534 mCi/mg, Dupont Co.) was added to 1 x 10^6/ml target cells which were subsequently incubated for 2 hours at room temperature. The cells were then washed 3 times with serum-free media and diluted to a volume of 1 x 10^5 cells/ml in RPMI 1640/10% calf serum. The effector spleen cells were washed once, resuspended and diluted to a concentration of 1 x 10^7 cells/ml of RPMI medium. 1:2 serial dilutions were made from this stock cell solution (5 x 10^6, 2.5 x 10^6 and 1.25 x 10^6 cells/ml). One hundred (100) μl of these effector cell solutions were aliquoted into a 96-well microliter flat bottom plate. One hundred (100) μl of target cell solution was added to each well. The resultant effector to target cell ratios were 100:1, 50:1, 25:1 and 12.5:1. In order to determine the spontaneous or maximum chromium release, respectively, target cells were mixed with either 100 μl of media alone or 1% Triton-X. The effector and target cells were then incubated at 37°C in a 5% CO_2 incubator for 5 hours. A 100 μl aliquot of supernatant was removed from each well, and the amount of ^{51}Cr release was measured in a gamma counter. The formula for calculation of the specific CTL release is below: 100 x [(experimental release - spontaneous release) / maximum release - spontaneous release)]. Note: maximum release was determined by lysis of target cells in 1% Triton X-100.

Example 13: CTL Assay Using Clinical HIV-1 Isolates

HeLa CD4+ cells expressing, mouse MHC-I were infected with HIV-1 clinical isolates and used as target cells in the CTL assay. The CTL assay was performed as described in Chada, *et al.*, *J. Virol.*, **1993**, *67*, 3409.

Example 14: T Cell Proliferation Assay

Assays were performed in triplicate. Splenocytes were isolated as discussed above, resuspended in RPMI 1640 and diluted to a concentration of 3.3×10^6 cells/ml. A 150 µl aliquot was immediately added to each well of a 96-well microtiter flat bottom plate. Fifty (50) µl of protein or peptide was added to each well to final concentrations of 10.0, 1.0 or 0.1 mg/ml. The cells were incubated at 37°C in a 5% $C0_2$ incubator for 3 days. One (1) µCi of tritiated thymidine was added to each well, and the cells were incubated overnight under the same conditions. The cells were harvested using automated cell harvester (Tomtec, Orange, CT) and the amount of incorporated tritiated thymidine was measured in a beta counter. In order to ensure that the cells were healthy, 5 mg/ml of PHA was used as a non-specific stimulator in a positive control sample.

Example 15: Transcomplementation Of vif Defective Proviral DNA With vif Genes From Maternal Samples

RD cells (1×10^6) were co-transfected with 10 µg of a vif defective proviral clone, p9l1, and 10 µg pCVif or vif expression plasmid from transmitter or non-transmitter subjects using lipofectin from Boehringer Mannheim (Indianapolis, IN). The co-transfected cells were washed after an 8 hour incubation and resuspended in DMEM media. Culture supernatant was collected after a 72 hour incubation, centrifuged to remove cell debris, passed through a 0.45 µm filter, and assayed for p24 production (Coulter Corporation). PBMCs (1×10^7) were infected with an amount of virus equivalent to 100 ng of p24 antigen. Virus-inoculated cells were incubated for 4-6 hours at 37°C and 5% $C0_2$, washed 3 times with PBS and resuspended in 10 ml of fresh RPMI 1640. An aliquot of the culture supernatant was collected every 3 days in order to quantitate virus production by measuring the amount of p24 antigen released into the medium.

Example 16: Characterization Of Viruses Isolated From Patients

The HIV-1 positive transmitter and non-transmitter mothers included in the present invention were selected from an AIDS cohort study. The mother and the non-transmitter mother are referred to as T1 and N1, respectively. The clinical status of the subjects and the replication kinetics of their viral isolates are presented in Table 2.

Uncultured lymphocytes from each subject were used in order to obtain wild-type sequences unmodified by *in vitro* selection conditions. In PBMC co-cultivation assays, T1 viral samples replicated very well in normal donor PBLs; whereas N1 viral samples did not replicate in either primary lymphocytes or macrophages.

Table 2

Subject	Clinical Stage	PCR	Virus Coculture in PBMC	Infection in CD4+ Cell Lines
Transmitter	Asymptomatic	+++	+++	+++
Non-Transmitter	Asymptomatic	++	---	---

Example 17: Sequence Variation Of *Vif* Gene In Vivo

In order to investigate the genetic variability of the *vif* gene in these subjects, ten clones from each subject were sequenced and computer-aligned by degree of homology. The nucleotide sequences were then translated into protein sequences. Deduced amino acid sequences were used in the final comparison, since not all nucleotide sequence chances resulted in amino acid sequence changes. The aligned amino acid sequences from these patients are shown in Figure 1. Clone numbers with the designations, 'T' and 'N' represent variants isolated from transmitter and non-transmitter mothers, respectively. Sequence alignment revealed that each subject had a unique and highly conserved set of sequences within their virus pool. Most of the nucleotide changes were point mutations which generally resulted in substitutions, versus duplications or insertions, within the protein sequence. Three clones encoded attenuated proteins. Clone T-42 had a 5 amino acid deletion at its 3' end due to a premature stop codon. Clone N-13 had two stop codons (positions 31 and 41) and clone T-4 had a single stop codon (position 77), each of which was introduced within a set of three nucleotides, keeping the reading frame intact 3' to the mutation. The fact that the majority (17 of 20) of the clones encode full-length sequences suggests that there are few defective *vif* genes present within these patients' viral pools. It is interesting to note that most of the *vif* point mutations are present in the 5' portion of the gene rather than in the 3' region.

Significant differences were found between clones at positions 20, 27, 31, 36, 37, 45, 60, 74, 127, 136, 140 and 150.

In order to determine the nature and the sequence variation of *vif* gene *in vivo*, we cloned and analyzed vif variants present in uncultured PBMCs from HIV-1 positive subjects. Analysis of 20 different *vif* sequences from two subjects (10 from each subject) revealed that *vif* is highly conserved (approximately 90%) within a particular patient at a given time point. Although, Wieland, et al. (*Virol.*, **1994**, *203*, 43) reported that the 3' portion of the *vif* gene is highly variable, the results of the present invention indicate that the 5' portion (aa 20-85) is more variable and the 3' portion is well-conserved. In support of the results herein, previous mutagenesis experiments have shown that the C terminus of vif (aa 171 to 192) is essential for stable association of vif with membranes. Goncalves, *et al.*, *J. Virol.*, **1994**, *68*, 704. Among the 20 sequences we analyzed, only two clones had premature stop codons indicating that 90% of *vif* genes isolated were intact *in vivo*. This result, along, with previously published data, suggests that a complete vif gene is essential for viral replication *in vivo*. Gabudza, *et al.*, *J. Virol.*, **1992**, *66*, 6489; *and* Sova, *et al.*, *J. Virol.*, **1995**, *69*, 2557.

The 20 deduced vif protein sequences from these clones exhibited 75% conservation (25% variation) over the entire (192 aa) length. In particular, two antigenic domains, aa 87-94 (IEWRKKRY) (SEQ ID NO: 24) and aa 172-178 (DRWNKPQ) (SEQ ID NO: 25), recognized by HIV-1 positive sera (Wieland, *et al.*, *AIDS Res. Human Retrovir.*, **1991**, *7*, 861) are well conserved in all 20 clones. The well-conserved nature of these two regions may be responsible for the cross antigenic properties exhibited by these clones. In addition, a sequence which is conserved in 34/38 lentivirus vif, SLQYLA (144-149)(SEQ ID NO: 26) (Oberste, *et al.*, *Virus Genes*, **1992**, *6*, 95), is also conserved in each of the 20 vif clones sequenced in the present invention. In previous studies, computer alignment analyses has shown that amino acids 21 to 30, 103 to 115 and 142 to 150 of vif are highly conserved among HIV-1, HIV-2 and SIV. Myers, *et al.*, *Human Retrovir. AIDS*, **1988**. Clones analyzed in the present invention, however, were generally conserved sequences within aa 103-115 and aa 142-150, but not within aa 2l-30. Vif protein has been characterized as a cysteine protease with Cys ll4 marking its active site and His 48 considered to be important for activity. Guy,

et al., *J. Virol.*, **1991**, *65*, 1325. In the sequences of the present invention, Cys 114, as well as Cys 133 (the only other cystine in vif) and His 48, were well conserved.

Phylogenetic tree analysis (data not shown) found 3 major families within the 20 patient clones. Ninety (90%) percent of N-derived clones formed a family and 80% of T-derived clones formed a family while the remaining clones, N-30, T-3 and T-38, exhibited greater diversity and formed a Separate group (data not shown). When distance comparison was performed, intrapatient variation between the transmitter clones was 12%, versus a variation of 10% between non-transmitter clones. The similarity between the subjects' variant clones and the established laboratory molecular clones, HIV_{SF-2}, HIV_{NL43} and HIV_{Zr6}, was also evaluated. The subject isolates shared a higher degree of homology with other clones within their transmitter status group than with any of the laboratory-maintained viral isolates. Based upon their sequence variation, 4 clones from each patient were selected for preliminary translation/immunization experiments (see below).

Phylogenetic tree analysis also illustrated that, in spite of intra-patient variation, clones from the transmitter and nontransmitter subjects clustered separately. *In vitro* transcription/translation of 8 constructs (four from each subject) resulted in the expression of a 23 KDa protein, except in the case of clone N-13 which has a premature stop codon. This suggests that the various mutations present in these vif constructs did not affect the expression kinetics and stability of the protein.

Example 18: Expression Of *Vif* Clones

In vitro transcription/translation was performed upon 5 clones from each group in order to assess their levels of vif expression. Results are presented in Fig. 2. The products from the *in vitro* translation reactions were immunoprecipitated with vif antiserum and subjected to gel electrophoresis. pCVif (full length vif from HIV-1 strain SF2) and p911 *(vif* mutant) provirus were used as a positive and negative control, respectively. In vitro translation with pCVif and each of the full length *vif* expression plasmids produced a 23 kDa protein; whereas clones p9ll and N-13 did not result a protein product of 23 kDa size, probably due to the presence of premature stop codons. Two (2) clones from each subject group were selected for further evaluation, based upon similar serological characteristics (data not shown). The patient clones selected as representatives from each croup were T-35 (from

transmitter) and N-15 (from non-transmitter). Each of these clones contain mutations characteristic of their particular group and represent the highest level of diversity within these groups. It is interesting to note that mutations within clone N-15 are dispersed throughout the full length gene; whereas mutations within clone T-35 are clustered at the 5' end of the gene.

Example 19: Induction Of Humoral Responses *In Vivo*

Specific anti-vif immune responses were apparent in sera collected from mice immunized with T-35, N-15 and pCVif expression plasmids, but not in sera from mice immunized by pcDNA3 vector alone. The induction of immune response correlated with DNA injection concentration, as well as the number and time interval between boosts. Sera from 4 mice injected with either 50 or 100 µg of *vif* DNA had specific reactivity to vif protein when measured by ELISA (Fig. 3). Induction of the humoral response was dose-and time-dependent. Injection of 50 µg of DNA induced an immune response detectable by ELISA at 15 days following the first injection. This response increased after subsequent boosts, reaching a maximum level 45 days after 2 boosts (Panel A). Injection with 100 µg of DNA induced a response that reached a maximum level only 28 days after a single boost (Panel B). In addition, the antibody response can be elevated 219 days after the three injections with a single boost of DNA (data not shown). The level of antibody response varied between vif clones. Most importantly, the non-transmitter clone, N-15, induced a higher serological response than the transmitter clone, T-38, or pCVif. This suggests that non-transmitter vif is capable of inducing a more efficient B-T helper dependent response than transmitter vif in this strain of mice.

Example 20: Induction Of Cellular Responses In Vivo Using Vaccinia Expressing Vif

Four mice, each immunized with one of the vif constructs, were given an additional boost 15 days after first injection. Two mice were subsequently sacrificed and their splenocytes were used in a cytotoxic T cell (CTL) assay. p815 cells infected with vif-expressing vaccinia were used as target cells. Non-specific lysis by splenocytes from vif DNA immunized and naive mice was measured using p815 cells infected with non-vif-expressing vaccinia as target cells. Specific target lysis is presented in Fig. 4. The level of

specific CTL activity varied between the *vif* constructs. Splenocytes from mice immunized with clone pCVif exhibited 45% lysis at a effector: target ratio of 100:1. Clones T-35 and N-15 exhibited 17 and 12% lysis, respectively, at the same ratio. These results clearly demonstrate that vif DNA immunization induces specific CTL responses. The differences in the levels of CTL activity induced by vif gene inoculation between the various patient clones may be due to mutations within the CTL epitopes expressed by vaccine targets or differences in immune responsiveness in this haplotype.

Example 21: Evaluation Of Cellular Responses *In Vivo* Using Human Targets Infected With A Clinical HIV-1 Isolate

In order to evaluate the ability of the vif clones to induce lysis of virally infected targets, we used HIV-1 infectable HeLa CD4/D^d cells which express both the CD4 receptor and the murine class I H-$2D^d$ restriction element, as targets in the CTL assay. These cells were infected with an HIV-1 isolate derived from a symptomatic AIDS patient for 7 days. Figure 5 (A-D) represents CTL assay results. Splenocytes obtained from mice injected with each of the DNA constructs exhibited vif-specific lysis. Clones T-35, N-15 and pCVif presented with 27, 26 and 24% lysis, respectively, at an effector:target ratio of 50:1. All three clones exhibited 20% lysis at a ratio of 25:1. This demonstrates that a cellular immune response against native HIV-1 isolates can be generated through genetic vaccination with vif expression vectors.

Example 22: Induction Of Antigen Specific T-Cell Proliferation

Specific T-cell proliferation responses against HIV-1 vif protein were also studied in DNA-immunized animals. Lymphocytes from vif-immunized mice demonstrated a significant proliferative response against rvif protein. Figure 6 illustrates the proliferation index of different vif constructs versus DNA injection concentrations. The results show that the MHC class II-dependent T_h (helper) cell response is dose dependent. For each construct, the stimulation index is almost 2-fold higher in mice injected with 100 μg of *vif* DNA than in mice injected with 50 μg of *vif* DNA. Comparison of the three different vif constructs also indicates that, at each injection concentration, clone T-35 induces a higher stimulation index than either N-15 or pCVif.

Example 23: Transcomplementation of HIV-1 Vif- Provirus With Vif Expression Plasmids

As expected, transient transfection of RD cells with HIV-1 (*vif*-) proviral DNA and vif expression plasmids did not reveal any differences in virus production between T-derived, N-derived or control plasmids (data not shown). Any differences in vif function would be demonstrated at the level of new infection. When rescued virus was used to infect primary lymphocytes, however, a significant difference was observed in virus pathogenesis between T- and N-derived and control plasmids (Table 3). The *vif*-negative proviral clone (p9l1) alone was unable to infect primary PBLs as cell-free virus. When trans-complemented virus (p9ll + pCVif) was used to infect the PBLs, infectivity was five-fold less than that of wild-type virus. In contrast, each of the T-derived clones tested were able to rescue the (*vif*-) mutant (approximately 100% positive virus control). However, none of the N-derived clones were able to efficiently infect PBLs as cell-free virus. Therefore, N-15 and similar N-derived clones were able to induce anti-HIV immune responses in mice in the absence of functionality.

Table 3

Samples	DNA Used to Derive Viruses for Infection	Amount of p24 Released (ng/ml)
Proviral Clone	pZr6	101,846
Vif Mutant	p911	60
Vif Mutant + pCVif	p911 + pCVif	22,679
Vif Mutant + Transmitter Clones	p911 + T1-40	21,896
	p911 + T1-37	17,230
	p911 + T1-35	19,470
	p911 + T1-38	81,570
Vif Mutant + Non-Transmitter Clones	p911 + N1-13	520
	p911 + N1-15	530
	p911 + N1-17	1,090
	p911 + N1-27	1,277
	p911 + N1-30	715

RD cells were transfected with 10 µg of pZr6, vif mutant p911, p911 and vif expression plasmids from different patient samples. Virus pools were prepared from supernatant collected 72 hours after transfection. Virus equivalent to 100 ng of p24 antigen was subsequently used to infect 10 x 10^6 PBMCs. Infection was monitored by p24 antigen production.

Example 24: Observations

N-derived clones were attenuated in their ability to transcomplement vif defective HIV-1 provirus. One of the clones analyzed, N-15, was also immunologically functional and capable of generating an immune response against wild-type HIV-1 virus. A non-functional yet immunogenic clone, such as N-15 in the present invention, could be an effective component of a genetic vaccine directed against HIV-1. It has been shown in the present invention that vif alone can generate an effective response against native HIV-1 virus *in vitro*. Such immunogens could be useful in a therapeutic setting to target the immune response against native vif expressing viruses. While it is likely that escape variants can occur viruses expressing defective vifs due to this selection might now exhibit attenuated *in vivo* growth kinetics. In a similar manner a prophylactic vaccine which includes vif could serve to both limit viral escape and contribute to lowering the viral set point during the early infection events.

SEQUENCE LISTING

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Weiner, David B.
University of Pennsylvania

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50 55 60

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35 40 45

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50 55 60

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65 70 75 80

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85 90 95

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His Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly His Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Arg Ala Gly His Ser Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Ile Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 12
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 12

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Thr Trp Asn Ser Leu Val Lys Tyr His Met Tyr Arg Ser
20 25 30

Lys Lys Ala Arg Glu Trp Phe Asn Arg His His Tyr His Arg Pro His
35 40 45

Pro Lys Val Ser Ser Glu Val His Ile Pro Leu Glu Asp Ala Arg Leu
50 55 60

Glu Ile Thr Thr Phe Trp Gly Leu His Thr Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly His Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Arg Ala Gly His Ser Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Ile Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 13
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 13

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Thr Trp Asn Ser Leu Val Lys Tyr His Met Tyr Arg Ser
20 25 30

Gln Lys Glu Arg Glu Trp Phe Asn Arg His His Tyr His Ser Pro His
35 40 45

Pro Glu Gln Ser Ser Thr Ala His Ile Pro Leu Val Asp Gly Arg Leu
50 55 60

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

Arg Gly His Arg Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Val Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly His Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Arg Ala Gly His Ser Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Ile Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 14
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 14

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Thr Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
35 40 45

Pro Lys Val Ser Ser Thr Ala His Ile Pro Leu Gly Asp Gly Arg Leu
50 55 60

Glu Lys Thr Ala Val Trp Ser Leu Gln Ala Gly Asp Gly Val Trp His
65 70 75 80

Arg Gly His Pro Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 9 0 95

Gln Val Asp Pro Asp Leu Val Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 15
<211> 191
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 15

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Arg Thr Trp Phe Ser Arg His His Tyr Gly Ser Pro His
35 40 45

Pro Lys Val Cys Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Val Ile Thr Thr Tyr Trp Ser Leu His Ala Gly Glu Asp Trp His Val
65 70 75 80

Gly Gln Arg Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr Gln
85 90 95

Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe Asp
100 105 110

Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg Val
115 120 125

Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser Leu
130 135 140

Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys Pro
145 150 155 160

Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys Pro
165 170 175

Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 16
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 16

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Thr Tyr Phe Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
35 40 45

Pro Asn Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Val Thr Thr Pro Tyr Trp Gly Leu His Gly Gly Glu Arg Asp Trp Tyr
65 70 75 80

Leu Ala Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 17
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 17

Met Glu Asn Arg Trp Glu Val Met Ile Val Trp Glu Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
35 40 45

Pro Lys Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Val Ile Thr Thr Tyr Trp Gly Leu His Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 18
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 17

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Asn Ala Lys Lys Trp Phe Tyr Arg His His Tyr Asp Ser Pro His
35 40 45

Pro Val Gln Ser Ser Thr Ala His Ile Pro Leu Gly Asp Gly Arg Leu
50 55 60

Gln Lys Ile Ala Phe Trp Ser Leu Asp Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Arg His Thr Met Asn Gly His
180 185 190

<210> 19
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 19

Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Asp Ser Pro His
35 40 45

Pro Lys Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Glu Thr Thr Thr Tyr Trp Gly Leu His Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

His Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 20
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 20
Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Thr Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
35 40 45

Pro Lys Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Val Ile Thr Thr Tyr Trp Gly Leu His Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Thr His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 21
<211> 188
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 21
Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Asn Arg His His Tyr Asp Arg Pro His
35 40 45

Pro Lys Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Glu Ile Thr Thr Phe Trp Gly Leu His Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Arg Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Thr His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly Thr Glu Gly Ala Ile Gln
180 185

<210> 22
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 22
Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Phe Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
35 40 45

Pro Lys Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Glu Ile Thr Thr Phe Trp Gly Leu His Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Gly Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Gly Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 23
<211> 192
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 23
Met Glu Asn Arg Trp Gln Val Met Ile Val Trp Gln Val Asp Arg Met
1 5 10 15

Arg Ile Arg Ala Trp Asn Ser Leu Val Lys His His Met Tyr Val Ser
20 25 30

Lys Lys Ala Lys Lys Trp Phe Tyr Arg His His Tyr Glu Ser Pro His
35 40 45

Pro Gln Val Ser Ser Glu Val His Ile Pro Leu Gly Asp Ala Arg Leu
50 55 60

Glu Ile Thr Thr Tyr Trp Gly Leu His Ala Gly Glu Arg Asp Trp His
65 70 75 80

Leu Gly Gln Gly Val Ser Ile Glu Trp Arg Lys Arg Arg Tyr Ser Thr
85 90 95

Gln Val Asp Pro Asp Leu Ala Asp Gln Leu Ile His Leu Tyr Tyr Phe
100 105 110

Asp Cys Phe Ser Glu Ser Ala Ile Arg Lys Ala Ile Leu Gly Tyr Arg
115 120 125

Val Ser Pro Arg Cys Glu Tyr Gln Ala Gly His Asn Lys Val Gly Ser
130 135 140

Leu Gln Tyr Leu Ala Leu Ala Ala Leu Ile Thr Pro Lys Lys Ile Lys
145 150 155 160

Pro Pro Leu Pro Ser Val Arg Lys Leu Thr Glu Asp Arg Trp Asn Lys
165 170 175

Pro Gln Lys Thr Lys Gly His Arg Gly Ser His Thr Met Asn Gly His
180 185 190

<210> 24
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 24
Ile Glu Trp Arg Lys Lys Arg Tyr
1 5

<210> 25
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 25
Asp Arg Trp Asn Lys Pro Gln
1 5

<210> 26
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 26
Ser Leu Gln Tyr Leu Ala
1 5

<210> 27
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 27

atggaaaaca gatggcaggt gattattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat tgatcaaaga aagctaggga atggttttat 120
tgacatcact atcaaagtcc tcatccaaaa gtaagttcag aagtacacat cccactagag 180
gatgctagat tggaaataac atcattttgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca cgtcgaccct 300
gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgg cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 28
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 28
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggttttat 120
agacatcact atcaaagtcc tcatccaaga gtaagttcag aagtacacat cccactagag 180
gatgctagat tggaaataac aacatattgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgc cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 29
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 29
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggttttat 120
agacatcact atcaaagtcc tcatccaaaa gtaagttcag aagtacacat cccactagag 180
gatgctagat tggaaataac aacatattgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300

gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgc cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 30
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 30
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaacata ccatatgtat agatcacaga aagctaggga atggtttaat 120
agacatcact atcacagtcc tcatccaaaa gtaagttcag aagtccacat cccactagag 180
gatgctagat tggcaatacc aacattttgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgc cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 31
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 31
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggttttat 120
agacatcact atcaaagtcc tcatccaaaa gtaagttcag aagtccacat cccactagag 180
gatgctagat tggaaataac aacatattgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca cgtcgaccct 300
gatctcgcag accacctaat tcatctgtgt tattttgatt gtctttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgc cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 32
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 32
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggttttat 120
agacatcact atcaaagtcc tcatccaaaa gtaagttcag aagtacacat cccactagag 180
gatgctagat tggtaataac aacatattgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca cgtagaccct 300
gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgg cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 33
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 33
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggttttat 120
agacatcact atcaaagtcc tcatccaaaa gtaagttcag aagtacacat cccactagag 180
gatgctagat tggtaataac aacatattgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gatctagcag accacctaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgg cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 34
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 34
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggttttat 120
agacatcact atcaaagtcc tcatccaaaa gtaagttcag aagtacacat cccactagag 180
gatgctagat tggtaataac aacattttgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca cgtagaccct 300
gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgc cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggtcaca gagggagcca tacaatgaat ggacactag 579

<210> 35
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

<400> 35
atggaaaaca gatggcaggt gatgattgtg tggcaggtag acaggatgag gattagaaca 60
tggaacagtt tagtaaaata ccatatgtat agatcaaaga aagctaggga atggtttaat 120
agacatcact atcaccgtcc tcatccaaaa gtaagttcag aagtccacat cccactagag 180
gatgctagat tggaaataac aacattttgg ggtctgcata caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gatctagcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggaca cagagttagt cctaggtgtg aatatcgagc aggacatagc 420
aaggtaggat cactacagta cttggcaata gcagcattaa taacaccaaa aaagataaag 480
ccacctttgc cgagtgtcag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aagggccaca gagggagcca tacaatgaat ggacactag 579

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<211> 584
<212> DNA
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<223> Description of Artificial Sequence: Novel Sequence

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tttatagaca tcactatcac agccctcatc cagaacaaag ttcaacagcc cacatcccgc 180
tagtggatgg tagattggaa aaaatagcag tttggagtct ggatacagga gatggcgtct 240
ggcacagggg gcatcgagtc tccatagaat ggaggaaaag gagatatagc acacaagtag 300
accctgatct agtagaccaa ctaattcatc tgtattattt tgattgtttt tcagaatctg 360
ctataagaaa agccatatta ggacacagag ttagtcctag gtgtgaatat cgagcaggac 420
atagcaaggt aggatcacta cagtacttgg caatagcagc attaataaca ccaaaaaaga 480
taaagccacc tttgccgagt gtcaggaaac tgacagagga tagatggaac aagccccaga 540
agaccaaggg ccacagaggg agccatacaa tgaatggaca ctag 584

<210> 37
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<223> Description of Artificial Sequence: Novel Sequence

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agacatcact atgaaagccc tcatccaaaa gtaagttcaa cagcccacat cccgctaggg 180
gatggtagat tggagaaaac agcagtttgg agtctgcagg caggagatgg agtctggcac 240
agggggcatc cagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gatttggtag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggata tagagttagt cctaggtgtg aataccaagc aggacataat 420
aaggtaggat ctctacagta cttggcacta gcagcattaa taacaccaaa gaagataaag 480
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aagggccaca gagggagcca tacaatgaat ggacactag 579

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<220>
<223> Description of Artificial Sequence: Novel Sequence

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agacatcact atggaagccc tcatccaaaa gtatgttcag aagtacacat cccactaggg 180
gatgctagat tggtgataac aacatattgg agtctgcatg caggagaatg agactggcat 240
gtgggtcaga gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gacttggcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360

agaaaagcca tattaggata tagagttagt cctaggtgtg aataccaagc aggacataat 420
aaggtaggat ctctacagta cttggcacta gcagcattaa taacaccaaa gaagataaag 480
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aagggccaca gagggagcca tacaatgaat ggacactag 579

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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

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gatgctagat tggtgacaac accatattgg ggtctgcatg gaggagaaag agactggtat 240
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aaggtaggat ctctacagta cttggcacta gcagcattaa taacaccaaa gaagataaag 480
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<220>
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cgacatcact atgacagccc tcatccagtc caaagttcaa cagcccacat cccgctaggg 180
gatggtagat tgcagaaaat agcattttgg agtctggatg caggagaaag agactggcat 240
ttgggtcagg gagtctccat agaatggagg aaaaggagat atagcacaca agtagaccct 300
gacctggcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggata tagagttagt cctaggtgtg aataccaagc aggacataat 420
aaggtaggat ctctacagta cttggcacta gcagcattaa taacaccaaa gaagataaag 480
ccacctttgc ctagtgtgag gaaactgaca gaggatagat ggaacaagcc ccagaagacc 540
aaggggcaca gagggaggca tacaatgaat ggacactag 579

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<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

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gacctggcag accaactaat tcatctgtat tattttgatt gtttttcaga atctgctata 360
agaaaagcca tattaggata tagagttagt cctaggtgtg aataccaagc aggacataat 420
aaggtaggat ctctacagta cttggcacta gcagcattaa taacaccaaa gaagataaag 480
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aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 43
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<213> Artificial Sequence

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gatgctagat tggtgataac aacatattgg ggtctgcatg caggagaaag agactggcat 240
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gacttggcag accaactaac tcatctgtat tattttgatt gtttttcaga atctgctata 360
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<220>
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<211> 579
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<220>
<223> Description of Artificial Sequence: Novel Sequence

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agaaaagcca tattaggata tagagttagt cctaggtgtg aataccaagc aggacataat 420
aaggtaggat ctctacagta cttgggacta gcagcattaa taacaccaaa gaagataaag 480
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aagggccaca gagggagcca tacaatgaat ggacactag 579

<210> 46
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Novel Sequence

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agacatcact atgaaagccc tcatccacaa gtaagttcag aagtacacat cccactaggg 180
gatgctagat tggagataac aacatattgg ggtctgcatg caggagaaag agactggcat 240
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aagggccaca gagggagcca tacaatgaat ggacactag 579

What is Claimed is:

1. An isolated, attenuated, non-functional *vif* protein.

2. The protein of claim 1 wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23.

3. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an attenuated, non-functional *vif* protein.

4. The nucleic acid molecule of claim 3 wherein said nucleic acid molecule comprises a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

5. The nucleic acid molecule of claim 3 wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46.

6. A pharmaceutical composition comprising a protein of claim 1 in a pharmaceutically acceptable carrier or diluent.

7. A pharmaceutical composition comprising a nucleic acid molecule of claim 3 in a pharmaceutically acceptable carrier or diluent.

8. A recombinant expression vector comprising a nucleic acid molecule of claim 3.

9. The recombinant expression vector of claim 8 wherein said nucleic acid molecule comprises a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

10. A host cell comprising a recombinant expression vector comprising a nucleic acid molecule of claim 3.

11. The host cell of claim 8 wherein said nucleic acid molecule comprises a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

12. A purified antibody directed against an attenuated, non-functional *vif* protein.

13. The antibody of claim 12 wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23.

14. A method of immunizing a mammal against a virus comprising administering to cells of said mammal, a nucleic acid molecule that comprises a nucleotide sequence that encodes an attenuated, non-functional *vif* protein, wherein said nucleic acid molecule is expressed in said cells.

15. The method of claim 14 wherein said nucleic acid molecule comprises a nucleotide sequence which encodes an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23.

16. The method of claim 14 wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46.

17. The method of claim 14 wherein said virus is selected from the group consisting of human immunodeficiency virus, feline immunodeficiency virus, bovine immunodeficiency virus, Visna virus, and simian immunodeficiency virus.

18. A plasmid comprising a nucleotide sequence encoding an isolated, attenuated, non-functional *vif* protein.

19. The plasmid of claim 18 wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23.

20. The plasmid of claim 18 wherein said nucleotide sequence is selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, 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, SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46.

CA 02304125 2000-03-15

SUBSTITUTE SHEET (RULE 26)


```
    1                                                  51                                                 100
N24 ---------- ---------- ------y--- r-----e--- ----q----- ---------e ----v----- ---t------ ---------- ------h---
N27 ---------- ---------- ------y--- r-----e--- ----q----- ---------e ----v----- ---t------ ---------- ------h---
N26 ---------- ---------- ------y--- r-----e--- ----q----- ---------e ----v----- ---t------ ---------- ----------
N15 ---------- ---------- ------y--- r-----e--- ----q----r ---------e ---------- ---t------ ---------- ----------
N17 ---------- ---------- ------y--- r-----e--- ----q----- ---------e ---------- ---t------ ---------- ----------
N23 ---------- ---------- ------y--- r-----e--- ----q----- ---------e ---------- ---t------ ---------- ------h---
N29 ---------- ---------- ------y--- r-----e--n ----hr---- ---------e ---------- ---t------ ---------- ----------
N13 -------i-- ---------- ------y--- *-----e--- *---q----- ---------e -------s-- ---t------ ---------- ------h---
N22 ---------- ---------- -----ty--- r-q---e--n ----h----- ---------e ----a-p--- ---t------ ---------- ----------
T39 ---------- ---------a ---------- -----kk--- ---------- ---------- ---------- ---a------ ---------- ------h---
T44 ---------- ---------a ---------- -----kk--- ---------q ---------- ---------- ---a------ ---------- ----------
T43 ---------- ---------a ---------- -----kk--- ---------- ---------- ---------- ---a------ ---------- ----------
T37 -----e---- -e-------a ---------- -----kk--- ---------- ---------- ----v----- ---a------ ---------- ----------
T40 ---------- ------t--a ---------- -----kk--- ---------- ---------- ----v----- ---a------ ---------- ----------
T35 ---------- ---------a --------i- f----kk--- ---------n ---------- ----vt-p-- ---g-----y -a------- ----------
 T4 ---------- ---------a ---------- ------t--s ----g----- -c-------- ----v----- s--a--*--- v--r------ ----------
 T3 ---------- ---------- ---------- -----kk--- ---------- ---ta----- -g---k-av- s-qa--gv-- r-hp------ ----------
T38 ---------- ---------a ---------- ---n-kk--- ---------v q--ta----- -g--qkia-- s-da------ ---------- ----------
T42 ---------- ---------a ---------- -----kk--n -----r---- ---------- ---------- ---a------ ---r------ ----------
Con MENRWQVMIV WQVDRMRIRT WNSLVKHHMY VSKKAR-WFY RHHYESPHPK VSSEVHIPLG DARLEITTYW GLH-GERDWH LGQGVSIEWR KRRYSTQVDP

101                                                151                                                194
N24 ---------- ---------- ------h--- -----r---s ---------i ---------- ---a------ ---------- ------.--- ---*
N27 ---------- ---------- ------h--- -----r---s ---------i ---------- ---------- ---------- ------.--- ---*
N26 ----h----- ---------- ------h--- -----r---s ---------i ---------- ---a------ ---------- ------.--- ---*
N15 ---------- ---------- ------h--- -----r---s ---------i ---------- ---------- ---------- ------.--- ---*
N17 ---------- ---------- ------h--- -----r---s ---------i ---------- ---------- ---------- ------.--- ---*
N23 ----h----- ---------- ------h--- -----r---s ---------i ---------- ---------- ---------- ------.--- ---*
N29 ---------- ---------- ------h--- -----r---s ---------i ---------- ---------- ---------- ------.--- ---*
N13 ---------- ---------- ------h--- -----r---s ---------i ---------- ---a------ ---------- ------.--- ---*
N22 ---------- ---------- ------h--- -----r---s ---------i ---------- ---------- ---------- ------.--- ---*
T39 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
T44 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
T43 ---------- --g------- ---------- ---------- -------g-- ---------- ---------- ---------- ------.--- ---*
T37 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
T40 ------t--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
T35 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
 T4 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
 T3 --v------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------.--- ---*
T38 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----r.--- ---*
T42 ------t--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --te-aiq*- dt..
Con DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGS-HTM NGH-
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FIGURE 1

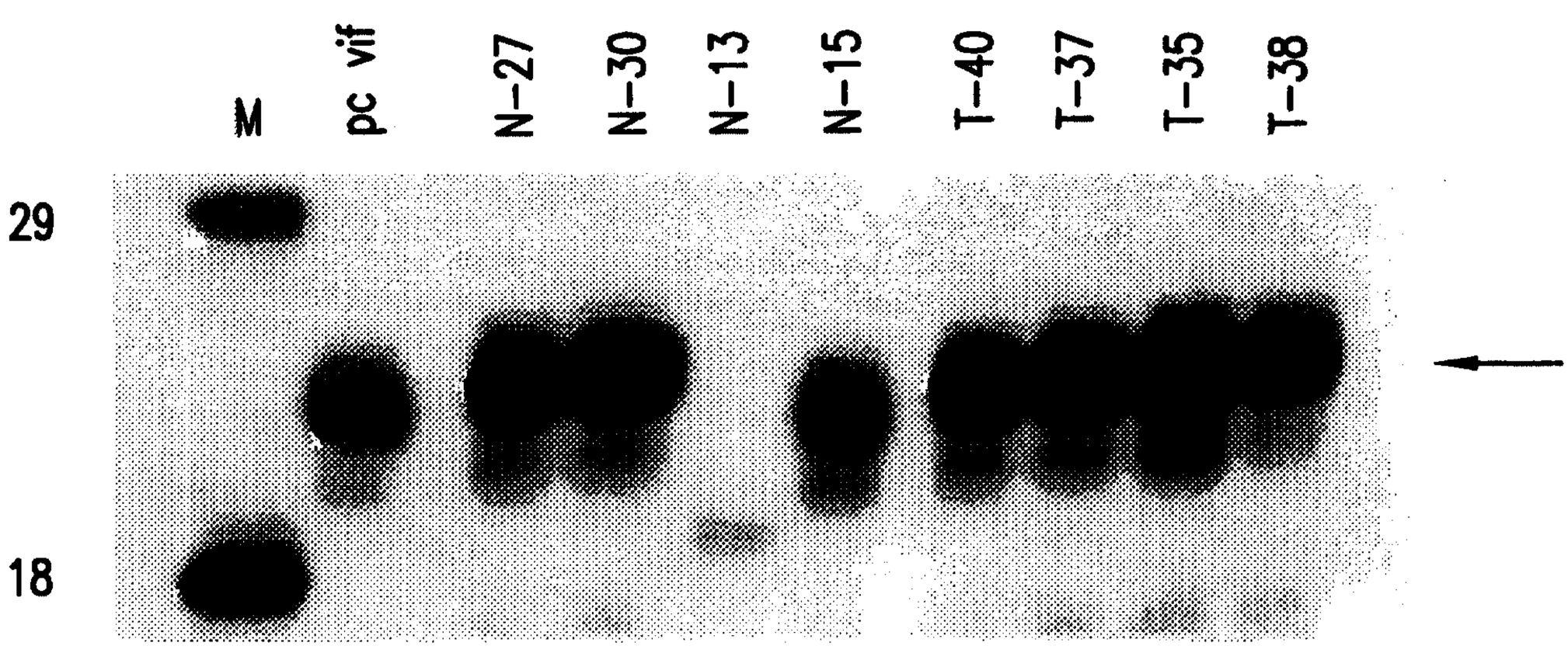


FIG.2

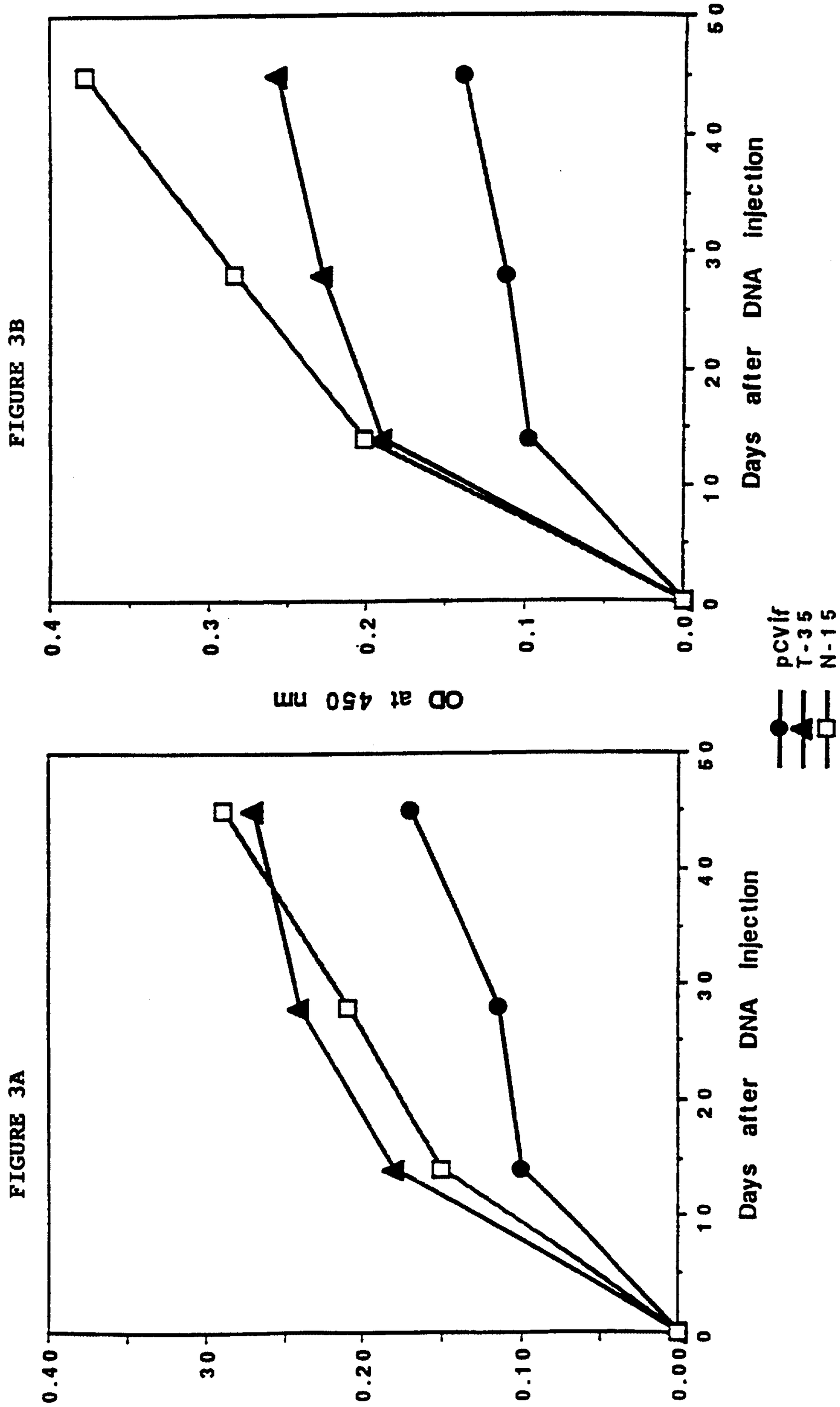


FIGURE 3B

FIGURE 3A

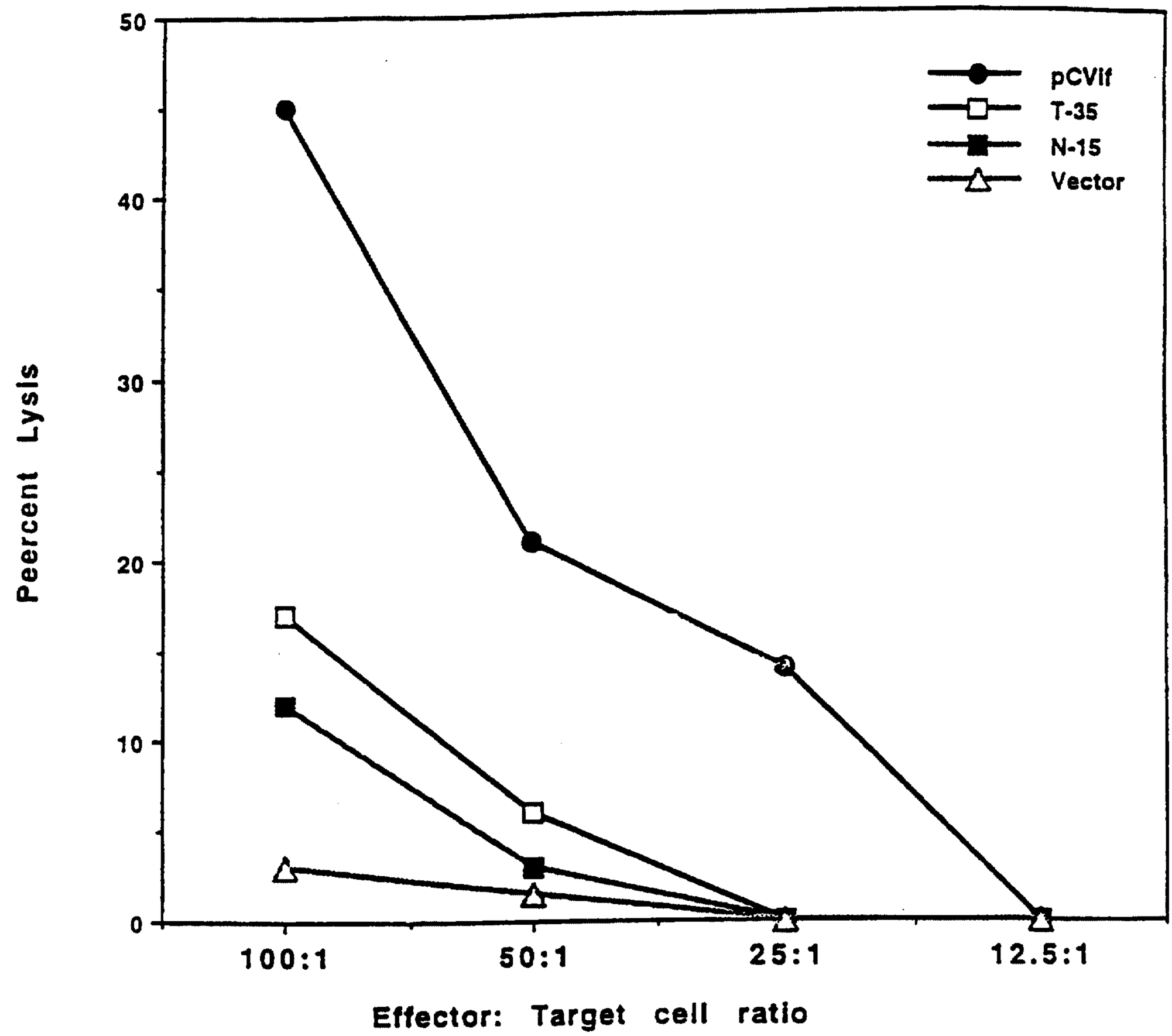
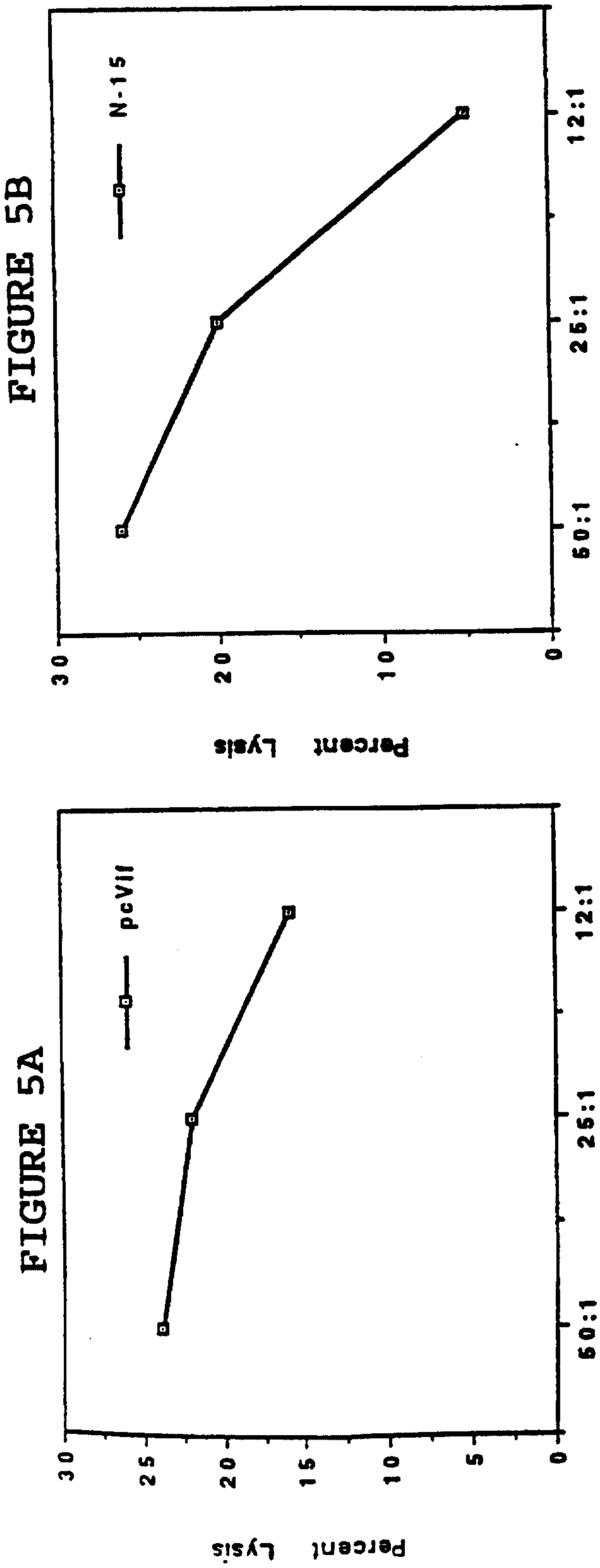

50
40
30
20
10
0
Peercent Lysis
pCVIf
T-35
N-15
Vector
100:1
50:1
25:1
12.5:1
Effector: Target cell ratio

FIGURE 4


FIGURE 5A
pcVII
Percent Lysis
Cell Ratio
12:1
25:1
50:1
0
10
20
30
5
15
25

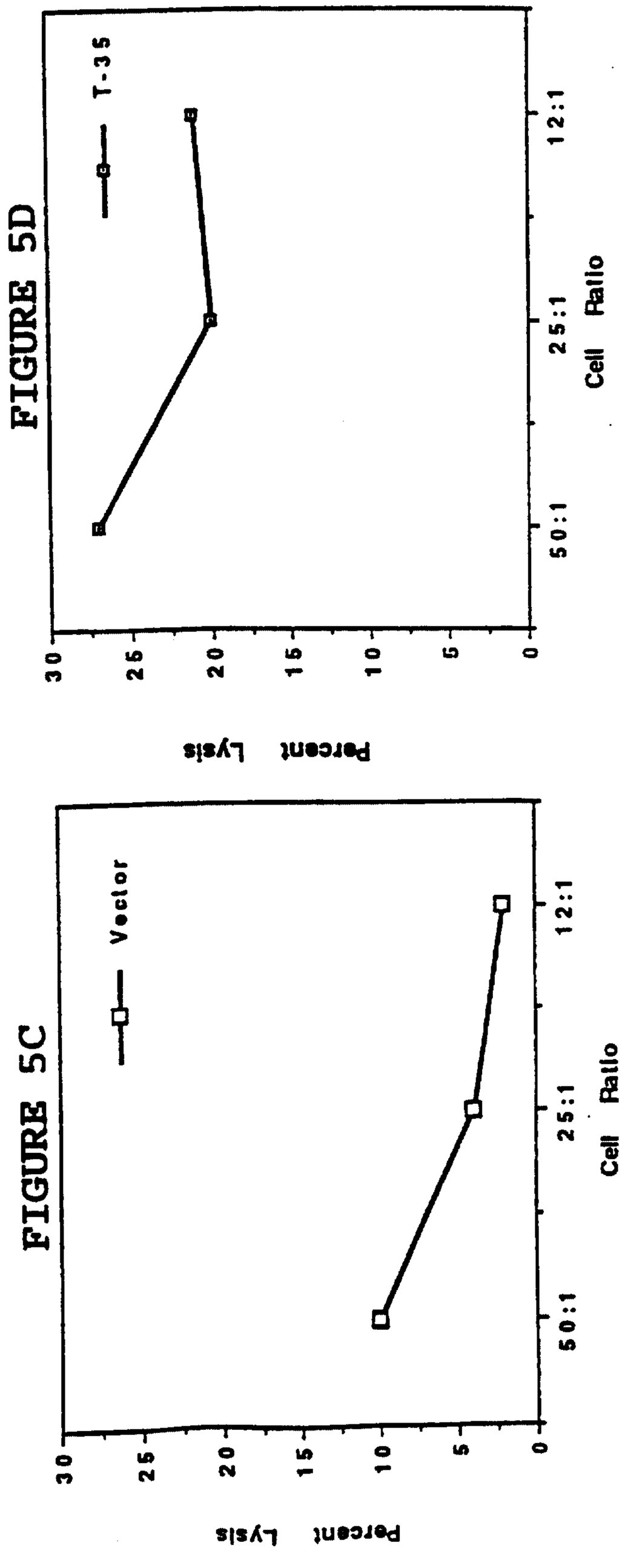
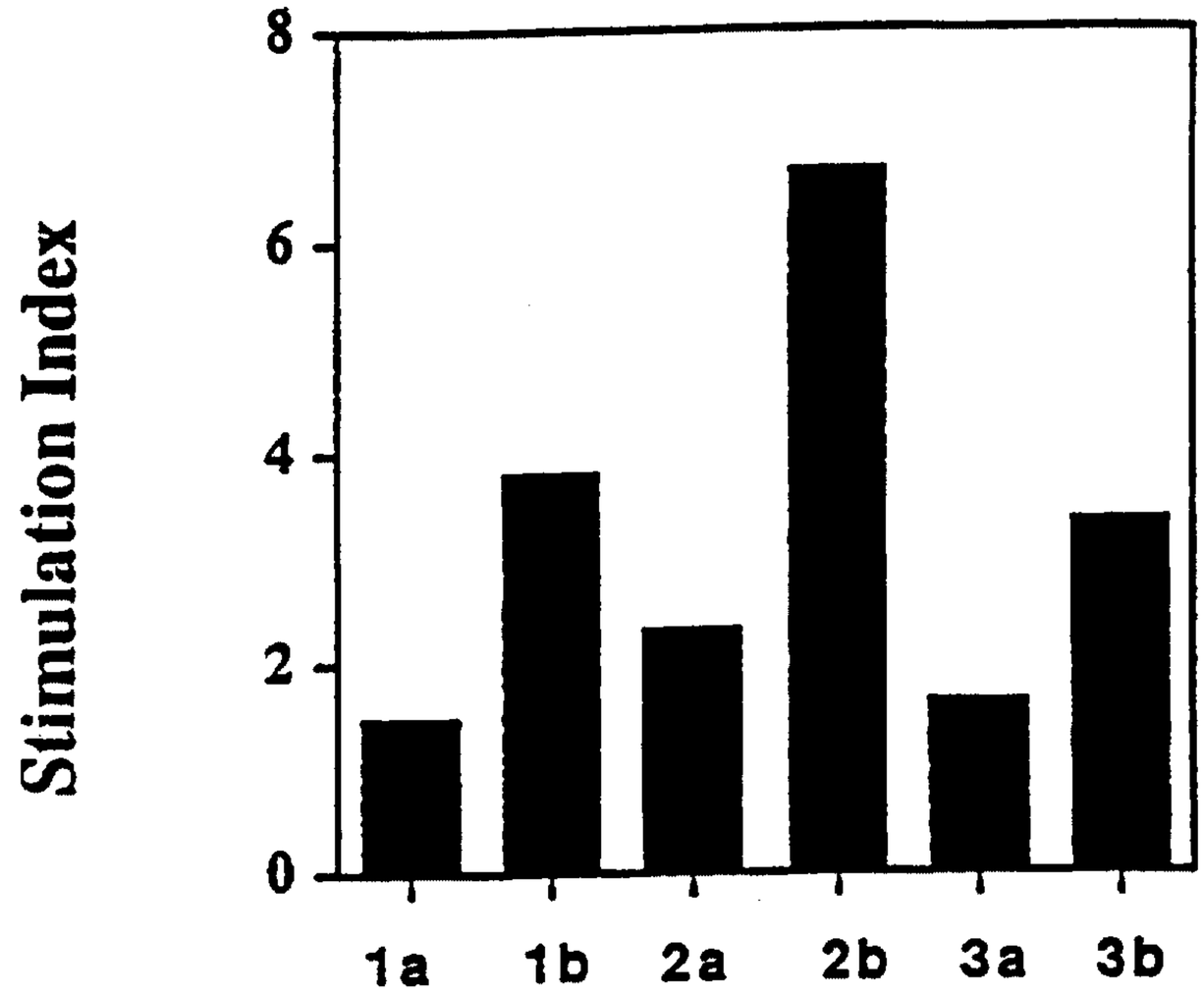

FIGURE 5B
N-15
Percent Lysis
Cell Ratio
12:1
25:1
50:1
0
10
20
30

FIGURE 5C
Vector
Percent Lysis
Cell Ratio
12:1
25:1
50:1
0
5
10
15
20
25
30

FIGURE 5D
T-35
Percent Lysis
Cell Ratio
12:1
25:1
50:1
0
5
10
15
20
25
30

T-cell proliferation



Vif expression constructs used

FIGURE 6

FIGURE 7A

1. Vif-N13.pep

MENRWQVIIV WQVDRMRIRT WNSLVKYHMY *SKKAREWFY *HHYQSPHPK

VSSEVHIPLE DARLEITSFW GLHIGERDWH LGQGVSIEWR KRRYSIHVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLASVRKLT EDRWNKPQKT KGHRGSHIMN GH*

2. Vif-N15.pep

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSKKAREWFY RHHYQSPHPR

VSSEVHIPLE DARLEITTYW GLHIGERDWH LGQGVSIEWR KRRYSTQVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

3. Vif-N17.pep

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSKKAREWFY RHHYQSPHPK

VSSEVHIPLE DARLEITTYW GLHIGERDWH LGQGVSIEWR KRRYSTQVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

FIGURE 7B

4. Vif-N22.pep

MENRWQVMIV WQVDRMRIRT WNSLVTYHMY RSQKAREWFN RHHYHSPHPK

VSSEVHIPLE DARLAIPTFW GLHIGERDWH LGQGVSIEWR KRRYSTQVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

5. Vif-N24.Pep

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSKKAREWFY RHHYQSPHPK

VSSEVHIPLE DARLVITTYW GLHIGERDWH LGQGVSIEWR KRRYSIHVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLASVRKLT EDRWNKPQKT KGHRGSHIMN GH*

6. Vif-N26.pep

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSKKAREWFY RHHYQSPHPK

VSSEVHIPLE DARLVITTYW GLHIGERDWH LGQGVSIEWR KRRYSIQVDP

DLADHLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLASVRKLT EDRWNKPQKT KGHRGSHIMN GH*

7. Vif-N27.pep

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSKKAREWFY RHHYQSPHPK

VSSEVHIPLE DARLVITTFW GLHIGERDWH LGQGVSIEWR KRRYSIHVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

FIGURE 7C

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

8. Vif-N29.pep

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSKKAREWFN RHHYHRPHPK

VSSEVHIPLE DARLEITTFW GLHIGERDWH LGQGVSIEWR KRRYSTQVDP

DLADQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

9. Vif-N30.

MENRWQVMIV WQVDRMRIRT WNSLVKYHMY RSQKEREWFN RHHYHSPHPE

QSSTAHIPLV DGRLEKIAVW SLDIGEGVWH RGHRVSIEWR KRRYSTQVDP

DLVDQLIHLY YFDCFSESAI RKAILGHRVS PRCEYRAGHS KVGSLQYLAI

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T3.pep

MENRWQVMIV WQVDRMRIRT WNSLVKHHMY VSKKAKKWFY RHHYESPHPK

VSSTAHIPLG DGRLEKIAVW SLQAGDGVWH RGHPVSIEWR KRRYSTQVDP

DLVDQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T35.pep

FIGURE 7D

MENRWQVMIV WQVDRMRIRA WNSLVKHHIY FSKKAKKWFY RHHYESPHPN

VSSEVHIPLG DARLVITTPYW GLHGGERDWY LAQGVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T37.pep

MENRWEVMIV WEVDRMRIRA WNSLVKHHMY VSKKAKKWFY RHHYESPHPK

VSSEVHIPLG DARLVITTYW GLHAGERDWH LGQGVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T38.pep

MENRWQVMIV WQVDRMRIRA WNSLVKHHMY VSKNAKKWFY RHHYDSPHPV

QSSTAHIPLG DGRLQKIAFW SLDAGERDWH LGQGVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGRHIMN GH*

Vif-T39.pep

MENRWQVMIV WQVDRMRIRA WNSLVKHHMY VSKKAKKWFY RHHYDSPHPK

VSSEVHIPLG DARLEITTYW GLHAGERDWH LGQGVSIEWR KRRYSIHVDP

FIGURE 7E

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T4.pep

MENRWQVMIV WQVDRMRIRA WNSLVKHHMY VSKKARIWFS RHHYGSPHPK

VCSEVHIPLG DARLVITTYW SLHAGE*DWH VGQRVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T40.pep

MENRWQVMIV WQVDRMTIRA WNSLVKHHMY VSKKAKKWFY RHHYESPHPK

VSSEVHIPLG DARLVITTYW GLHAGERDWH LGQGVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T42.pep

MENRWQVMIV WQVDRMRIRA WNSLVKHHMY VSKKAKKWFN RHHYDRPHPK

VSSEVHIPLG DARLEITTFW GLHAGERDWH LGQRVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGTEGAIQ*

FIGURE 7F

Vif-T43.pep

MENRWQVMIV WQVDRMRIRA WNSLVKHHMF VSKKAKKWFY RHHYESPHPK

VSSEVHIPLG DARLEITTFW GLHAGERDWH LGQGVSIEWR KRRYSIQVDP

DLADQLIHLY YFGCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLGL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

Vif-T44.pep

MENRWQVMIV WQVDRMRIRA WNSLVKHHMY VSKKAKKWFY RHHYESPHPQ

VSSEVHIPLG DARLEITTYW GLHAGERDWH LGQGVSIEWR KRRYSIQVDP

DLADQLIHLY YFDCFSESAI RKAILGYRVS PRCEYQAGHN KVGSLQYLAL

AALITPKKIK PPLPSVRKLT EDRWNKPQKT KGHRGSHIMN GH*

FIGURE 8A

N13 (SEQ ID NO:27)

ATGGAAAACAGATGGCAGGTGATTATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATTGATCAAAGAAAGCTAGGGAATGGTTTTAT
TGACATCACTATCAAAGTCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGAG
GATGCTAGATTGGAAATAACATCATTTTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACACGTCGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGGCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N15 (SEQ ID NO:28)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTTAT
AGACATCACTATCAAAGTCCTCATCCAAGAGTAAGTTCAGAAGTACACATCCCACTAGAG
GATGCTAGATTGGAAATAACAACATATTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N17 (SEQ ID NO:29)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTTAT
AGACATCACTATCAAAGTCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGAG
GATGCTAGATTGGAAATAACAACATATTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N22 (SEQ ID NO:30)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAACATACCATATGTATAGATCACAGAAAGCTAGGGAATGGTTTAAT
AGACATCACTATCACAGTCCTCATCCAAAAGTAAGTTCAGAAGTCCACATCCCACTAGAG
GATGCTAGATTGGCAATACCAACATTTTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

FIGURE 8B

N23 (SEQ ID NO:31)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTTAT
AGACATCACTATCAAAGTCCTCATCCAAAAGTAAGTTCAGAAGTCCACATCCCACTAGAG
GATGCTAGATTGGAAATAACAACATATTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACACGTCGACCCT
GATCTCGCAGACCACCTAATTCATCTGTGTTATTTTGATTGTCTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N24 (SEQ ID NO:32)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTTAT
AGACATCACTATCAAAGTCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGAG
GATGCTAGATTGGTAATAACAACATATTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACACGTAGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGGCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N26 (SEQ ID NO:33)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTTAT
AGACATCACTATCAAAGTCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGAG
GATGCTAGATTGGTAATAACAACATATTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GATCTAGCAGACCACCTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGGCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N27 (SEQ ID NO:34)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTTAT
AGACATCACTATCAAAGTCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGAG
GATGCTAGATTGGTAATAACAACATTTTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACACGTAGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGTCACAGAGGGAGCCATACAATGAATGGACACTAG

FIGURE 8C

N29 (SEQ ID NO:35)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATAGATCAAAGAAAGCTAGGGAATGGTTTAAT
AGACATCACTATCACCGTCCTCATCCAAAAGTAAGTTCAGAAGTCCACATCCCACTAGAG
GATGCTAGATTGGAAATAACAACATTTTGGGGTCTGCATACAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GATCTAGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGACATAGC
AAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGATAAAG
CCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

N30 (SEQ ID NO:36)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAGGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAATACCATATGTATTGATCAAAGAAAAGAAAGAAAGGGAATGGT
TTTATAGACATCACTATCACAGCCCTCATCCAGAACAAAGTTCAACAGCCCACATCCCGC
TAGTGGATGGTAGATTGGAAAAAATAGCAGTTTGGAGTCTGGATACAGGAGATGGCGTCT
GGCACAGGGGGCATCGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAG
ACCCTGATCTAGTAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTG
CTATAAGAAAAGCCATATTAGGACACAGAGTTAGTCCTAGGTGTGAATATCGAGCAGGAC
ATAGCAAGGTAGGATCACTACAGTACTTGGCAATAGCAGCATTAATAACACCAAAAAAGA
TAAAGCCACCTTTGCCGAGTGTCAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGA
AGACCAAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

T3 (SEQ ID NO:37)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAACA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGAAAGCCCTCATCCAAAAGTAAGTTCAACAGCCCACATCCCGCTAGGG
GATGGTAGATTGGAGAAAACAGCAGTTTGGAGTCTGCAGGCAGGAGATGGAGTCTGGCAC
AGGGGGCATCCAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GATTTGGTAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTTAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

T4 (SEQ ID NO:38)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAGGACATGGTTTTCT
AGACATCACTATGGAAGCCCTCATCCAAAAGTATGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGTGATAACAACATATTGGAGTCTGCATGCAGGAGAATGAGACTGGCAT
GTGGGTCAGAGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACTTGGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

FIGURE 8D

T35 (SEQ ID NO:39)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATTTATTTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGAAAGCCCTCATCCAAACGTAAGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGTGACAACACCATATTGGGGTCTGCATGGAGGAGAAAGAGACTGGTAT
CTGGCTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACCTGGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

T37 (SEQ ID NO:40)

ATGGAAAACAGATGGGAGGTGATGATTGTGTGGGAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGAAAGCCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGTGATAACAACATATTGGGGTCTGCATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACCTGGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

T38 (SEQ ID NO:41)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAACGCTAAGAAATGGTTTTAT
CGACATCACTATGACAGCCCTCATCCAGTCCAAAGTTCAACAGCCCACATCCCGCTAGGG
GATGGTAGATTGCAGAAAATAGCATTTTGGAGTCTGGATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACCTGGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGGCACAGAGGGAGGCATACAATGAATGGACACTAG

T39 (SEQ ID NO:42)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGACAGCCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGAGATAACAACATATTGGGGTCTGCATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACACGTAGACCCT
GACCTGGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

FIGURE 8E

T40 (SEQ ID NO:43)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGACGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGAAAGCCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGTGATAACAACATATTGGGGTCTGCATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACTTGGCAGACCAACTAACTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

T42 (SEQ ID NO:44)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAAGAAATGGTTTAAT
AGACATCACTATGACCGCCCTCATCCAAAAGTAAGTTCAGAAGTCCACATCCCACTAGGG
GATGCTAGATTGGAGATAACAACATTTTGGGGTCTGCATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGCGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACTTGGCAGACCAACTAACTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCACAGAGGGAGCCATACAATGAATGGACACTAG

T43 (SEQ ID NO:45)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTTTGTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGAAAGCCCTCATCCAAAAGTAAGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGAGATAACAACATTTTGGGGTCTGCATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACCTGGCAGACCAACTAATTCATCTGTATTATTTTGGTTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGGACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG

T44 (SEQ ID NO:46)

ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAGAGCA
TGGAACAGTTTAGTAAAACACCATATGTATGTTTCAAAGAAAGCTAAGAAATGGTTTTAT
AGACATCACTATGAAAGCCCTCATCCACAAGTAAGTTCAGAAGTACACATCCCACTAGGG
GATGCTAGATTGGAGATAACAACATATTGGGGTCTGCATGCAGGAGAAAGAGACTGGCAT
TTGGGTCAGGGAGTCTCCATAGAATGGAGGAAAAGGAGATATAGCACACAAGTAGACCCT
GACCTGGCAGACCAACTAATTCATCTGTATTATTTTGATTGTTTTTCAGAATCTGCTATA
AGAAAAGCCATATTAGGATATAGAGTTAGTCCTAGGTGTGAATACCAAGCAGGACATAAT
AAGGTAGGATCTCTACAGTACTTGGCACTAGCAGCATTAATAACACCAAAGAAGATAAAG
CCACCTTTGCCTAGTGTGAGGAAACTGACAGAGGATAGATGGAACAAGCCCCAGAAGACC
AAGGGCCACAGAGGGAGCCATACAATGAATGGACACTAG