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(54) Titre : VECTEURS D'EXPRESSION A BASE DE POXVIRUS CONTENANT DES INSERTS HETEROLOGUES
DERIVES DE LENTIVIRUS

(54) Title: POXVIRUS-BASED EXPRESSION VECTORS CONTAINING HETEROLOGOUS INSERTS DERIVED FROM
LENTIVIRUSES

(57) Abrégé/Abstract:

Recombinants containing and expressing lentivirus, retrovirus or immunodeficiency virus DNA and methods for making and using the same are disclosed and claimed. In an exemplified embodiment, attenuated recombinant viruses containing DNA encoding a feline immunodeficiency virus epitope such as an antigen, as well as methods and compositions employing the viruses, expression products therefrom, and antibodies generated from the viruses or expression products, are disclosed and claimed. The recombinants can be NYVAC or ALVAC recombinants. The DNA can encode at least one of: Env, Gag, Pol, or combinations thereof such as Gag and Pol or protease or Env, Gag and Pol or protease. The recombinants and gene products therefrom and antibodies generated by them have several preventive, therapeutic and diagnostic uses. DNA from the recombinants are useful as probes or, for generating PCR primers or for immunization. The immunogenicity and protective efficacy of immunization protocols involving ALVAC-FIV and priming with a recombinant canarypox virus ALVAC-FIV followed by a booster immunization with inactivated FIV-infected celled vaccine (ICV) was evaluated against-FIV challenge in cats and the protocol was shown to effectively induce FIV-specific protective immune responses. Further, it was found that immunized cats were fully protected from an initial challenge with a slightly heterologous FIV strain (50CID₅₀) and were partially protected from a second challenge with a distinctly heterologous FIV strain (75CID₅₀) given eight months after the initial challenge without any intervening booster.

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(21) International Application Number: PCT/US97/20430 (22) International Filing Date: 7 November 1997 (07.11.97) (30) Priority Data: 08/746,668 14 November 1996 (14.11.96) US (71) Applicant: VIROGENETICS CORPORATION [US/US]; Rensselaer Technology Park, 465 Jordan Road, Troy, NY 12180 (US). (72) Inventors: TARTAGLIA, James; 7 Christina Drive, Schenec- tady, NY 12303 (US). PAOLETTI, Enzo; 297 Murray Av- enue, Delmar, NY 12054 (US). (74) Agent: SMITH, G., Kenneth; McDonnell, Boehnen, Hulbert & Berghoff, 300 South Wacker Drive, Chicago, IL 60606 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: POXVIRUS-BASED EXPRESSION VECTORS CONTAINING HETEROLOGOUS INSERTS DERIVED FROM LENTIVIRUSES (57) Abstract Recombinants containing and expressing lentivirus, retrovirus or immunodeficiency virus DNA and methods for making and using the same are disclosed and claimed. In an exemplified embodiment, attenuated recombinant viruses containing DNA encoding a feline immunodeficiency virus epitope such as an antigen, as well as methods and compositions employing the viruses, expression products therefrom, and antibodies generated from the viruses or expression products, are disclosed and claimed. The recombinants can be NYVAC or ALVAC recombinants. The DNA can encode at least one of: Env, Gag, Pol, or combinations thereof such as Gag and Pol or protease or Env, Gag and Pol or protease. The recombinants and gene products therefrom and antibodies generated by them have several preventive, therapeutic and diagnostic uses. DNA from the recombinants are useful as probes or, for generating PCR primers or for immunization. The immunogenicity and protective efficacy of immunization protocols involving ALVAC-FIV and priming with a recombinant canarypox virus ALVAC-FIV followed by a booster immunization with inactivated FIV-infected celled vaccine (ICV) was evaluated against FIV challenge in cats and the protocol was shown to effectively induce FIV-specific protective immune responses. Further, it was found that immunized cats were fully protected from an initial challenge with a slightly heterologous FIV strain (50CID₅₀) and were partially protected from a second challenge with a distinctly heterologous FIV strain (75CID₅₀) given eight months after the initial challenge without any intervening booster.		

**POXVIRUS-BASED EXPRESSION VECTORS CONTAINING
HETEROLOGOUS INSERTS DERIVED FROM LENTIVIRUSES**

STATEMENT OF POSSIBLE GOVERNMENT RIGHTS

Some work reported herein may have been supported by a NIH/NIAID grant (R01-AI30904) and a Virogenetics Corp./University of Florida collaborative grant. The government may have certain rights (without prejudice or admission).

FIELD OF THE INVENTION

The present invention relates: to certain
5 product(s) from lentivirus, retrovirus and/or
immunodeficiency virus, e.g., HIV, SIV, EIAV, BIV, FIV,
comprising certain epitope(s) of interest, preferably
Env, Gag, Pol, and accessory gene products, e.g. Tat,
Rev, more preferably of Gag and Pol or Env, Gag and Pol
10 and most preferably Gag and protease; to certain nucleic
acid molecule(s), e.g., RNA, DNA, encoding the
product(s); to a vector, preferably a mammalian vector
system, comprising the nucleic acid molecule(s) and
preferably expressing the product(s) as exogenous to the
15 vector; to the product(s) obtained or obtainable from
expression by the vector; to immunological, immunogenic
and/or vaccine compositions comprising the vector and/or
the product(s); to methods for preparing the product(s);
to methods for preparing the vector; to methods for
20 preparing the compositions; and to methods for using the
product(s), vector and compositions, including methods
for obtaining an immunological response such as by
immunization regimens wherein the product(s), vector
and/or compositions are administered alone or in a
25 prime/boost configuration with inactivated lentivirus,
retrovirus or recombinant subunit preparations, e.g., in
a prime/boost configuration with an inactivated infected
cell vaccine or immunological or immunogenic composition
(ICV).

30 The invention especially relates to recombinant
immunological, immunogenic or vaccine compositions and
their utility in stimulating a response, such as
providing protection against a lentivirus challenge
exposure, including exposure to a heterologous strain.
35 The recombinant composition is preferably comprised of a
mammalian vector system expressing lentivirus gene
products used in effective immunization regimens alone or

in a prime/boost configuration with inactivated lentivirus preparations (e.g., ICV) or recombinant subunit preparations.

Several documents are referenced in this application. Full citation to these documents is found at the end of the specification immediately preceding the claims or where the document is mentioned.

10 BACKGROUND OF THE INVENTION

The patent and scientific literature includes various mammalian vector systems such as mammalian virus-based vector systems and mammalian DNA-based vector systems, and how to make and use these vector systems, for instance for cloning of exogenous DNA and expression of proteins, as well as uses for such proteins and uses for products from such proteins.

For instance, recombinant poxvirus (e.g., vaccinia, avipox virus) and exogenous DNA for expression in this viral vector system can be found in U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941, 5,338,683, 5,494,807, 5,503,834, 4,722,848, 5,514,375, U.K. Patent GB 2 269 820 B, WO 92/22641, WO 93/03145, WO 94/16716, PCT/US94/06652, and U.S.

Patent No. 5,833,975. See generally Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996; Moss, "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996.

Baculovirus expression systems and exogenous DNA for expression therein, and purification of recombinant proteins therefrom can be found in Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.) (see, e.g., Ch.18 for influenza HA expression, Ch.19 for recombinant protein purification techniques),

Smith et al., "Production of Human Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and
 5 Regulated Expression of *Escherichia coli* B-Galactosidase in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573 (Skin test and test kit for AIDS, discussing baculovirus expression systems containing
 10 portion of HIV-1 env gene, and citing EP Patent publication No. 265785).

U.S. Patent No. 4,769,331 relates to herpesvirus as a vector. See also Roizman, "The function
 15 of herpes simplex virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996; Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA
 20 93:11313-11318, October 1996. Epstein-Barr virus vectors are also known. See Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996. Further, there are alphavirus-based vector systems. See generally Frolov et
 25 al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996.

There are also poliovirus and adenovirus vector systems (see, e.g., Kitson et al., J. Virol. 65, 3068-3075, 1991; Grunhaus et al., 1992, "Adenovirus as cloning
 30 vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993; Ballay et al. EMBO Journal, vol. 4, p. 3861-65; Graham, Tibtech 8, 85-87, April, 1990; Prevec et al., J. Gen Virol. 70, 429-434). See also U.S.

Patent Nos. 6,156,567 and 6,090,393.
 35 (adenovirus vector system, preferably CAV2) and PCT WO91/11525 (CAV2 modified to contain a promoter-gene sequence within the region from the SmaI site close to

the end of the inverted terminal repeat region up to the promoter for the early region 4 (E4)).

There are also DNA vector systems. As to transfecting cells with plasmid DNA for expression therefrom, reference is made to Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561. As to direct injection of plasmid DNA as a simple and effective method of vaccination against a variety of infectious diseases reference is made to Science, 259:1745-49, 1993. See also McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996.

In 1983, human immunodeficiency virus type 1 (HIV1) was identified as the causative agent of AIDS and was subsequently classified into the lentivirus subfamily of the retrovirus family (Hardy, 1990). Other members of the lentivirus subfamily are equine infectious anemia virus (EIAV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), Simian immunodeficiency virus (SIV) and HIV-2. Much attention within the field of medical virology has been focused on the AIDS pandemic caused by infection with HIV. This lentivirus system has been scrutinized with respect to its molecular biology, immunobiology and pathogenesis in an effort to develop safe and effective vaccines and antiviral therapies. To date, HIV, as well as other lentiviral vaccine studies using different vaccine types have encountered varying degrees of success (Heeney et al., 1994; Daniel et al., 1992; Fultz et al., 1992; Girard et al., 1991; Issel et al., 1992). Further, knowledge is still lacking on the relevance of specific HIV immune responses on vaccine efficacy in humans. Thus, after many years, despite a massive, worldwide effort, an effective HIV1 vaccine is still not available.

Infection of cats with feline immunodeficiency virus (FIV) causes persistent infection and AIDS-like immunosuppressive diseases similar to the HIV infection. As such, FIV infection of cats provides a model for
5 investigating lentivirus immunopathogenicity and vaccine development (Pedersen et al., 1987; Johnson et al., 1994). Similar to HIV, heterogeneity exists, such that multiple FIV subtypes exist (Sadora et al., 1994; Okada et al., 1994). Indeed, like HIV, FIV strains have been
10 classified into four subtypes (A-D) based on genetic differences predominantly in the env and, to a lesser extent gag coding regions.

Thus, while inactivated whole FIV vaccines and inactivated FIV-infected cell vaccines (ICV) have
15 obtained protection against homologous and slightly heterologous FIV (Hosie et al., 1995; Johnson et al., 1994; Yamamoto et al., 1991, 1993), these same vaccines failed to induce protective immunity against distinctly heterologous FIV strains of other subtypes such that
20 induction of protective immunity against a broad range of FIV subtypes may call for a modified or different vaccine approach. This obviously raises concerns relevant to vaccine development. It must also be noted that the FIV prevalence in the cat population is greater than HIV is
25 in man (Verschoor et al., 1996). The development of an FIV vaccine or immunogenic composition is not only useful in providing a model for an HIV vaccine or immunogenic composition but is also therefore of importance from a veterinary health perspective.

30 More particularly, from the previous FIV studies (Hosie et al., 1995; Johnson et al., 1994; Yamamoto et al., 1991, 1993) it was observed that only cats with significant FIV Env-specific serum reactivity were likely to be protected against homologous challenge
35 exposure. In no case were vaccine-administered animals lacking such a response observed to be protected against FIV challenge (Johnson et al., 1994; Yamamoto et al.,

1991, 1993). Together, these results coupled to the observations, to date, that subunit immunogens have not been shown to elicit a protective immune response in target species bring to the forefront several important points relevant to the state-of-the-art for FIV and lentivirus, vaccine development in general. One exception perhaps is with the simian immunodeficiency virus (SIV)/macaque system where certain recombinant subunit preparations (including vaccinia-based recombinants) or combinations of these recombinant subunits have conferred, at least, partial protection from SIV challenge exposure (Hu *et al.*, 1992; 1994; 1995). This data is somewhat limited in scope since complete protection from infection was not observed and challenge studies were not performed with a distinctly heterologous SIV strain. Moreover, no level of protection was afforded by recombinant subunits devoid of an SIV Env component (Hu *et al.*, 1994).

Relevant to FIV vaccine development, no subunit based vaccine candidate has been taught or suggested; there is no teaching as to how to develop a subunit vaccine; and, it is not obvious as to how to develop a subunit-based vaccine candidate.

Secondly, a different or perhaps modified approach, as compared to the inactivated conventional vaccines, needs to be developed to afford protection against heterologous strains (Hosie *et al.*, 1995; Johnson *et al.*, 1994).

Lastly, Env-specific immune responses in protective immunity may be important (Johnson *et al.*, 1994; Yamamoto *et al.*, 1991, 1993). Indeed, in Flynn *et al.*, "ENV-specific CTL Predominate in Cats Protected from Feline Immunodeficiency Virus Infection by Vaccination," *The Journal of Immunology*, 1996, 157:3658-3665, at 3664 the authors conclude "that FIV Env-specific CTL may be more effective in protective immunity to FIV infection of

domestic cats" such that "future vaccine strategies should be aimed at eliciting both humoral and cell-mediated immune responses that are long-lived, recognize appropriate epitopes on the viral envelope glycoprotein, and are targeted to tissues known to sequester virus."

It can thus be appreciated that provision of a feline immunodeficiency virus recombinant subunit immunogenic, immunological or vaccine composition which induces an immunological response against feline immunodeficiency virus infections when administered to a host, e.g., a composition having enhanced safety such as NYVAC- or ALVAC- based recombinants containing exogenous DNA coding for an FIV epitope of interest, such as of FIV Env, Gag, or Pol, especially in an immunogenic configuration, or any combination thereof, for instance, FIV Gag-protease, Gag-Pol, or Gag and a portion of Pol (such as a portion of Pol including protease) or all of Env, Gag and Pol or a portion of Pol, in combination, would be a highly desirable advance over the current state of technology. Further, use of such recombinants or compositions containing such recombinants in a prime-boost regimen, e.g., wherein the recombinant composition is used in an initial immunization and a subsequent immunization is with an inactivated FIV, or ICV, or other recombinant subunit preparation would be a highly desirable advance over the current state of technology.

And more generally, it can thus be appreciated that provision of a lentivirus, retrovirus or immunodeficiency virus recombinant subunit immunogenic, immunological or vaccine composition which induces an immunological response against the lentivirus, retrovirus or immunodeficiency virus infections when administered to a host, e.g., a composition having enhanced safety such as NYVAC- or ALVAC- based recombinants containing exogenous DNA coding for a lentivirus, retrovirus, or immunodeficiency virus epitope of interest, such as Env, Gag, or Pol, especially in an immunogenic configuration,

or any combination thereof, for instance, Gag-protease, Gag-Pol or Gag and a portion of Pol (such as a portion including protease) all of Env, Gag and Pol or a portion of Pol, in combination such as Env, Gag-protease, in
5 combination, would be a highly desirable advance over the current state of technology. Further, use of such recombinants or compositions containing such recombinants in a prime-boost regimen, e.g., wherein the recombinant composition is used in an initial immunization and a
10 subsequent immunization is with an inactivated lentivirus, retrovirus or immunodeficiency virus, or ICV, or other recombinant subunit preparation, such as a respective inactivated virus, ICV or other recombinant subunit preparation would be a highly desirable advance
15 over the current state of technology (As to "respective", if the recombinant is, for example an FIV recombinant, inactivated FIV or an FIV ICV preparation may be "respective").

OBJECTS AND SUMMARY OF THE INVENTION

20 It is therefore an object of the invention to provide certain product(s) from lentivirus, retrovirus and/or immunodeficiency virus, e.g., HIV, SIV, EIAV, BIV, FIV, Visna virus, carpine arthritis-encephalitis virus, comprising certain epitope(s) of interest, preferably
25 Env, Gag, Pol or epitopes thereon, with optional accessory functions or proteins or epitope(s) of interest thereon e.g. Tat and/or Rev, more preferably Gag and Pol, or Env, Gag and Pol, or Gag and a portion of Pol, or Env, Gag and a portion of Pol, especially such a portion
30 including protease, and most preferably Gag and protease, or Env, Gag and protease, or epitopes thereon, with optional accessory functions or proteins, e.g., Tat and/or Rev or other such functions/proteins, or epitopes thereon. Other accessory functions or proteins which can
35 be included in the product(s) or epitope(s) of interest include any or all of net, vpu, vit, vpr, and vpx or epitope(s) thereon, inter alia; see Trono, D., Cell,

82:189-192, July 28, 1995. Such accessory functions or proteins may be considered non-envelope functions or proteins which can be included in the product(s) or epitope(s) of interest, e.g. for induction of a cellular
5 response. For instance, for a given lentivirus, retrovirus or immunodeficiency virus pathogen, that pathogen's accessory function(s) or protein(s) or epitope(s) thereon can be included; for example, the product(s) could thus include Gag-Pro plus accessory
10 function(s) or protein(s) or epitope(s) thereon.

It is an additional object of the invention to provide certain nucleic acid molecule(s), e.g., RNA, DNA, encoding the product(s), e.g., encoding certain epitope(s) of interest such as Gag and protease or all of
15 Env, Gag and Pol.

It is a further object of the invention to provide a vector, preferably a mammalian vector system, comprising the nucleic acid molecule(s) and preferably expressing the product(s) as exogenous to the vector,
20 e.g., a poxvirus, baculovirus, herpesvirus, Epstein-Barr, alphavirus, poliovirus, adenovirus or DNA vector system.

It is a further object of the invention to provide the product(s) obtained or obtainable from expression by the vector.

25 It is yet a further object of the invention to provide an immunological, immunogenic and/or vaccine composition comprising the vector and/or the product(s).

It is still another object of the invention to provide methods for preparing the product(s).

30 It is yet another object of the invention to provide methods for preparing the vector.

It is even still a further object of the invention to provide methods for preparing the compositions.

35 And it is a further object of the invention to provide methods for using the product(s), vector and compositions, including methods for obtaining an

immunological response such as by immunization regimens wherein the product(s), vector and/or compositions are administered alone or in a prime/boost configuration with inactivated lentivirus, retrovirus or recombinant subunit
5 preparations, e.g., in a prime/boost configuration with an inactivated infected cell vaccine or immunological or immunogenic composition (ICV), such as a respective ICV.

The present invention thus relates to
10 recombinant immunological, immunogenic or vaccine compositions and their utility in eliciting a response such as by providing protection against lentivirus, retrovirus or immunodeficiency virus challenge exposure in a target species.

15 More in particular, the invention relates to a mammalian vector system for the insertion and expression of foreign genes for use as safe immunization vehicles to elicit a response such as a protective immune response against lentiviruses, retroviruses or immunodeficiency
20 viruses.

In accord with the herein objects, the invention accordingly relates to a mammalian vector system, which expresses gene products (e.g., a gene product including an epitope of interest) of a
25 lentivirus, retrovirus or immunodeficiency virus such as EIAV, FIV, BIV, HIV, or SIV, with feline immunodeficiency virus (FIV) presently preferred; and, the invention relates to immunogenic and/or immunological and/or vaccine compositions which induce an immunological and/or
30 protective response against a lentivirus, retrovirus or immunodeficiency virus such as EIAV, FIV, BIV, HIV, or SIV exposure when administered to the target host, e.g., FIV and a feline, such as a domesticated cat or kitten.

In one aspect, in furthermore of the herein
35 objects, the present invention comprises a mammalian vector (e.g., poxvirus, baculovirus, herpesvirus, Epstein-Barr, alphavirus, poliovirus, adenovirus or DNA

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vector system, preferably a poxvirus) expressing a lentivirus, retrovirus or immunodeficiency virus epitope of interest, e.g., EIAV, FIV, BIV, HIV, or SIV, preferably FIV; and, the epitope of interest is preferably Gag/protease. The
5 vector is useful in the protection of the target species (e.g., feline) against a highly homologous challenge exposure; and accordingly, the invention encompasses an immunological, immunogenic or vaccine composition comprising the vector and optionally an acceptable carrier or diluent.

10 More particularly, in one aspect, the present invention relates to a vector comprising DNA encoding at least one Feline Immunodeficiency Virus (FIV) protein or immunogenic fragment thereof, characterized in that the vector is a poxvirus, adenovirus, or herpesvirus and to use
15 of this vector for treatment of FIV infection in an animal in need of such treatment. The present invention further relates to a composition for inducing an immunological response comprising this vector in admixture with a suitable carrier and to a method for expressing a gene product in a
20 cell cultured *in vitro* comprising introducing into the cell this vector. The present invention further relates to a commercial package comprising this vector together with instructions for treating Feline Immunodeficiency Virus (FIV) infection.

25 In another aspect, in accordance with the herein objects, the present invention comprises a method for inducing an immunological response, preferably a protective response comprising administering the vector or composition comprising the vector to a host. The method can be an
30 immunizing regimen, e.g., priming with the vector or composition comprising the vector (and expressing the lentivirus, retrovirus or immunodeficiency virus (e.g., FIV) epitope(s) of interest gene products) and boosting with a

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respective lentivirus, retrovirus or immunodeficiency subunit preparation (e.g., FIV inactivated whole cell (ICV) preparation) or with a respective lentivirus, retrovirus or immunodeficiency recombinant subunit preparation (e.g., FIV epitope(s) of interest from isolating such from expression of a recombinant containing exogenous nucleic acid molecule(s) encoding the same) to elicit an immunological response such as conferring protection to the host (e.g., cats) against homologous and heterologous lentivirus, retrovirus or immunodeficiency virus isolates.

It is an additional object of this invention to provide a recombinant poxvirus antigenic, vaccine or immunological composition having an increased level of safety compared to known recombinant poxvirus vaccines.

15 It is a further object of this invention to provide a modified vector for expressing a gene product

in a host, wherein the vector is modified so that it has attenuated virulence in the host.

It is another object of this invention to provide a method for expressing a gene product in a cell
5 cultured *in vitro* using a modified recombinant virus or modified vector having an increased level of safety.

These and other objects and advantages of the present invention will become more readily apparent after consideration of the following.

10 In a further aspect, the present invention relates to a vector, preferably a modified recombinant virus having inactivated virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety. The functions can be non-essential,
15 or associated with virulence (e.g., essential). The virus is advantageously a poxvirus, particularly a vaccinia virus or an avipox virus, such as fowlpox virus and canarypox virus. The vector which is preferably a modified recombinant virus can include, within an
20 essential or nonessential region of the virus genome, a heterologous DNA sequence which encodes an antigen or epitope derived from a lentivirus, retrovirus or immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus, such as,
25 e.g., Env, Gag, Pol, accessory functions (e.g. Tat, Rev), or any combination thereof, such as Gag-Pol or Env, Gag, and Pol or Gag and a portion of Pol or Env, Gag and a portion of Pol, such as a portion of Pol including protease, or Gag-protease or Env, Gag, and protease.

30 In another aspect, the present invention relates to an antigenic, immunological, immunogenic or vaccine composition or a therapeutic composition for inducing an antigenic or immunological or protective response in a host animal such as a feline, e.g.,
35 domesticated cat or kitten, inoculated with the composition, the composition can include a carrier and an inventive vector which is preferably a modified

recombinant virus having inactivated nonessential virus-encoded genetic functions so that the recombinant virus has attenuated virulence and enhanced safety; or the expression product of such a vector or modified
5 recombinant virus. The virus used in the composition (or for expressing a product for use in a composition) according to the present invention is advantageously a poxvirus, particularly a vaccinia virus or an avipox virus, such as fowlpox virus and canarypox virus. The
10 modified recombinant virus can include, within an essential or nonessential region of the virus genome, a heterologous DNA sequence which encodes an antigenic protein, e.g., an epitope of interest derived from a lentivirus, retrovirus or immunodeficiency virus, e.g.,
15 EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus, such as an antigen, e.g., Env, Gag, protease, or any combination thereof, such as Gag-protease or Env, Gag, and protease.

In yet another aspect, the present invention
20 relates to a method for inducing an antigenic, immunological, immunogenic, vaccine (protective), and/or therapeutic response in a host animal such as a feline, e.g., domesticated cat or kitten, for instance, a host animal in need of such a response, comprising
25 administering an amount of the aforementioned inventive composition effective to obtain the response, either alone or as part of a prime-boost regimen (e.g., administering the inventive composition or administering either or both of an inactivated lentivirus, retrovirus
30 or immunodeficiency virus or ICV or IWV either before or after administering the inventive composition).

In a further aspect, the present invention relates to a method for expressing a gene product in a cell in vitro by introducing into the cell an inventive
35 vector, such as a modified recombinant virus having attenuated virulence and enhanced safety. The vector or modified recombinant virus can include, within a

- nonessential or essential region of the virus genome, a heterologous DNA sequence which encodes an epitope of interest such as an antigenic protein, e.g. derived from a retrovirus, lentivirus or immunodeficiency virus, e.g.,
- 5 EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus, such as, e.g., Env, Gag, Pol, accessory functions (e.g. Tat, Rev), or any combination thereof, such as Gag-Pol, or Env, Gag and Pol, or Gag and a portion of Pol or Env, Gag or a portion of Pol wherein
- 10 the portion can include protease, or Gag-protease or Env, Gag, protease. The gene product can be harvested from the cells, or the cells can then be reinfused directly into an animal or used to amplify specific reactivities for reinfusion (Ex vivo therapy).
- 15 Thus, in a specific further aspect, the present invention relates to a method for expressing a gene product in a cell cultured *in vitro* by introducing into the cell a vector or preferably a modified recombinant virus having attenuated virulence and enhanced safety.
- 20 The vector or modified recombinant virus can include, within an essential or nonessential region of the virus genome, a heterologous DNA sequence which encodes an epitope of interest or an antigenic protein, e.g., derived from a lentivirus, retrovirus, or
- 25 immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus, such, e.g., Env, Gag, Pol, accessory functions (e.g. Tat, Rev), or any combination thereof, such as Gag-Pol, or Env, Gag and Pol, or Gag and a portion of Pol, or Env,
- 30 Gag and a portion of Pol, wherein the portion can include a protease, or Gag-protease or Env, Gag, protease. The product can then be administered to a host to stimulate a response.

- Antibodies can be raised by compositions
- 35 including the inventive vectors or recombinants or expression products of the inventive vectors or recombinants. The antibodies raised can be useful in a

host for the prevention or treatment of a lentivirus, retrovirus or immunodeficiency virus such as feline immunodeficiency virus. The antibodies or the expression products of the inventive vectors or recombinants can be
5 used in diagnostic kits, assays or tests to determine the presence or absence in a sample such as sera of lentivirus, retrovirus or immunodeficiency virus, e.g., feline immunodeficiency virus, or antigens thereof or antibodies thereto or of recombinants of the present
10 invention. Accordingly, an aspect of the invention involve the antibodies, diagnostic kits, assays, or tests.

In a still further aspect, the present invention relates to a modified recombinant virus having
15 nonessential or essential virus-encoded genetic functions inactivated therein so that the virus has attenuated virulence, and wherein the modified recombinant virus further contains DNA from a heterologous source in an essential or nonessential region of the virus genome.
20 The DNA can code for an antigen or epitope of interest of a lentivirus, retrovirus or immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus, e.g., Env, Gag, Pol, accessory functions, or any combination thereof, such as Gag-Pol or
25 Env, Gag and Pol, or Gag and a portion of Pol or Env, Gag and a portion of Pol, wherein the portion of Pol can include protease, or Gag-protease or Env, Gag, protease. In particular, the genetic functions are inactivated by deleting or disrupting an open reading frame encoding a
30 virulence factor, e.g., an essential region, or by utilizing naturally host restricted viruses, especially naturally host restricted viruses displaying attenuated virulence from having been serial passages and/or plaque purification (with or without subsequent passages). The
35 virus used according to the present invention is advantageously a poxvirus, particularly a vaccinia virus

or an avipox virus, such as fowlpox virus and canarypox virus.

Advantageously, the open reading frame is selected from the group consisting of J2R, B13R + B14R, 5 A26L, A56R, C7L - K1L, and I4L (by the terminology reported in Goebel et al., 1990a,b); any combination thereof and, preferably the combination thereof. In this respect, the open reading frame comprises a thymidine kinase gene, a hemorrhagic region, an A type inclusion 10 body region, a hemagglutinin gene, a host range gene region or a large subunit, ribonucleotide reductase; or, any combination thereof and preferably the combination thereof. The modified Copenhagen strain of vaccinia virus is identified as NYVAC (Tartaglia et al., 1992). 15 NYVAC and NYVAC variations have essential regions deleted or disrupted therein, and for a subsequent publication to Tartaglia et al., 1992 which like Tartaglia et al., 1992 relates to deletion of essential regions of vaccinia virus (and therefore relates to NYVAC, NYVAC variations, 20 or viruses taught by or obvious from viruses of Tartaglia et al., 1992 such as NYVAC and NYVAC variations) e.g., NYVAC.1, NYVAC.2, reference is made to PCT WO 95/30018. With respect to NYVAC and NYVAC variations, reference is also made to U.S. Patents Nos. 5,364,773 and 5,494,807. 25 However, other vaccinia virus strains, such as the COPAK strain, can also be used in the practice of the invention.

In another preferred embodiment the vector is an attenuated canarypox virus, such as a canarypox virus 30 which is not a mixed population. For instance, a Rentschler vaccine strain which was attenuated through more than 200 serial passages on chick embryo fibroblasts, a master seed therefrom was subjected to four successive plaque purifications under agar, from 35 which a plaque clone was amplified through five additional passages. Such a canarypox is called ALVAC.

Results of restriction digests of plaque derived from Kanapox is as follows: Genomic DNA isolated from plaque isolates number 1, 4, and 5 cloned from canarypox virus (Kanapox). DNA from these plaques and from the uncloned canarypox virus was digested with restriction enzymes HindIII, BamHI and EcoRI and run on a 0.8% agarose gel. The gel was stained with ethidium bromide and photographed under UV light (see Fig. 1).

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The restriction profiles show distinct differences between the various plaque isolates. Plaque 1 was chosen as ALVAC (CP_{pp}).

15 In the restriction patterns of the uncloned canarypox virus several submolar bands can be observed. However, when plaques cloned, these submolar bands become molar species in at least some of the plaque-cloned isolates. This indicates that the uncloned canarypox virus (Kanapox) represents a mixture of genomic variants which differ in restriction profiles.

Thus, ALVAC is different from, has unique properties over, and is superior to Kanapox; and therefore, ALVAC is a preferred vector in the practice of the invention. In particular, the Rentschler strain canarypox virus (Kanapox), from restriction analyses, represents a mixture of viral variants; that is, Kanapox, from which ALVAC was derived, was a mixed population. It is not unprecedented for a vaccine preparation, such as Kanapox, to contain multiple variants. ALVAC is not a mixed population. As such, ALVAC has several unique properties which are not shared by Kanapox, for instance:

ALVAC has a uniform genetic background. This property provides ALVAC with consistency; a unique feature of being useful for preparing vector-based vaccines. Consistency is useful for quality control and

regulatory considerations. This property of consistency of ALVAC provides ALVAC with the ability to pass quality control and regulatory considerations, i.e., be useful in the development of vector-based vaccines with predicted
5 genetic properties.

Biological consistency is controlled using ALVAC to derive recombinants. Kanapox does not provide biological consistency. Indeed, Kanapox cannot consistently provide an effective recombinant product.
10 Biological consistency and a consistently effective recombinant product are useful; for instance, for a consistent biological profile with respect to virulence, with regard to virus/host interactions, and ultimately for use as an immunization vehicle. ALVAC achieves
15 biological consistency and consistently effective recombinant products. When Kanapox is used in deriving recombinants, there is no control over the virus background into which the foreign gene is inserted; and therefore, the properties of the resultant recombinant
20 remain in question (cf. studies with vaccinia virus which illustrate that not all vaccinia genetic backgrounds are equivalent as immunization vehicles). ALVAC provides certainty with respect to its virus background, its properties related to virulence, and its functioning as
25 an immunization vehicle.

Although the present invention has primarily been described using a canarypox virus (ALVAC)-based vector, it should be understood that invention herein also resides in the expression of specific lentivirus,
30 retrovirus or immunodeficiency virus gene products and their utility for conferring an immune response such as a protective immune response. Hence, the invention also relates to alternative mammalian vector systems. Examples of such vector systems include other poxviruses,
35 adenoviruses, herpesviruses, alphavirus-based systems, bacterial expression systems, and DNA-based immunogen formulations.

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Although the present invention has primarily been described using the lentivirus FIV, it should be understood that invention herein also resides in the expression of functional homologs of the FIV gene products from other lentivirus and retrovirus and immunodeficiency virus systems, e.g., Env, Gag/protease (i.e., Env, Gag and Pol or a portion of Pol, or Gag and Pol or a portion of Pol wherein the portion of Pol can include protease (without Env), or Env, Gag, protease or Gag-protease (without Env) or Env, Gag, Pol or a portion of Pol and accessory functions (e.g. Tat, Rev) or Gag, Pol or a portion of Pol wherein the portion of Pol can include protease and accessory functions (without Env) or Env, Gag protease and accessory functions or Gag-protease and accessory functions (without Env), of EIAV, FIV, BIV, HIV, or SIV, *inter alia*). Hence, the invention relates to other lentivirus systems including human immunodeficiency virus -1, -2 (HIV-1, -2), bovine immunodeficiency virus (BIV), equine infectious anemia virus (EIAV), as well as other mammalian lentiviruses.

In another aspect, the invention provides use, for vaccinating a feline to induce a protective immune response against multiple subtypes of feline immunodeficiency virus (FIV), of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and a recombinant canarypox virus that comprises and expresses in vivo in a feline, a nucleic acid molecule that encodes one or more proteins selected from the group consisting of FIV Protease, FIV Env, the modified variant 97TM of FIV Env, and FIV Gag.

In another aspect, the invention provides use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response against multiple

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subtypes of feline immunodeficiency virus (FIV), of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and a recombinant canarypox virus that comprises and expresses in vivo in a feline, a
5 nucleic acid molecule that encodes one or more proteins selected from the group consisting of FIV Protease, FIV Env, the modified variant 97TM of FIV Env, and FIV Gag.

In another aspect, the invention provides use, for vaccinating a feline to induce a protective immune response
10 against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, a nucleic acid molecule encoding the FIV Env protein.

15 In another aspect, the invention provides use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an
20 ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, a nucleic acid molecule encoding the FIV Env protein.

In another aspect, the invention provides use, for vaccinating a feline to induce a protective immune response
25 against multiple subtypes of FIV, of an immunogenic composition comprising: an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env, Gag, and Protease proteins.

In another aspect, the invention provides use, in
30 the preparation of a medicament for vaccinating a feline to induce a protective immune response against multiple

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subtypes of FIV, of an immunogenic composition comprising: an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env, Gag, and Protease proteins.

5 In another aspect, the invention provides use, for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that
10 comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Gag and FIV Protease proteins.

 In another aspect, the invention provides use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response against multiple
15 subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Gag and FIV Protease proteins.

20 In another aspect, the invention provides use, for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that
25 comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env modified variant 97TM, FIV Gag and FIV Protease proteins.

 In another aspect, the invention provides use, in the manufacture of a medicament for vaccinating a feline to
30 induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a

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pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env modified variant 97TM, FIV Gag and FIV Protease proteins.

5 In another aspect, the invention provides a kit comprising: the immunogenic composition as described above; and instructions for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV.

10 In another aspect, the invention provides the immunogenic composition as described above, for the vaccination of a feline to induce a protective immune response against multiple subtypes of FIV.

15 In another aspect, the invention provides a vector comprising exogenous DNA encoding at least one FIV epitope, wherein the vector is a canarypox virus.

20 In another aspect, the invention provides the vector as described above, wherein the canarypox virus is the ALVAC strain, or is a Rentschler vaccine strain obtained by (a) attenuation of a Rentschler vaccine strain through more than 200 serial passages on chick embryo fibroblasts, (b) subjecting a master seed therefrom to four successive plaque purifications under agar, and (c) amplifying a plaque clone through five additional passages.

25 These and other embodiments are disclosed or are obvious from and encompassed by the following detailed description.

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DEPOSITS

The following have deposited with the ATCC under the terms of the Budapest Treaty.

	<u>Material</u>	<u>Accession Number</u>	<u>Deposit Date</u>
5	<u>ALVAC</u>	_____	<u>NOV. 14, 1996</u>
	Plasmid MM 138 (pMM138)	_____	<u>NOV. 14, 1996</u>
	<u>(containing FIV env,gag/pro)</u>		
	Plasmid MM 129 (pMM129)	_____	<u>NOV. 14, 1996</u>
	<u>(containing FIV gag/pro)</u>		

10 The invention thus comprehends nucleic acid molecules, including encoding product(s) having sequences as in the Deposited Material, as well as nucleic acid molecules having substantial homology thereto (e.g., at least 85% homology).

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings, incorporated herein by reference, in which:

FIG. 1 shows the results of plaque purifying Kanapox, as described above;

FIG. 2 shows the nucleotide sequence of FIV env from Rhone Merieux (SEQ ID NO:1) (The FIV env start codon is at position 1 and the stop codon is at position 2569. Plasmid ptg6184, containing the FIV env coding sequence, was from Rhone Merieux(Lyon, France). The FIV env coding sequence in ptg6184 was sequenced and the following differences with the sequence below were observed: position 1218 T is G in ptg6184 changing phe to leu; position 1220 G to A changes gly to glu; and position 2201 C to A change ala to glu);

FIG. 3 shows the nucleotide sequence of FIV gag/pol coding sequences from Rhone Merieux (SEQ ID NO:2) (The gag start codon is at position 1 and the gag stop codon is at position 1414. The ribosomal frameshift site is near position 1255. The frameshift is -1 in relation to the gag open reading frame. The frameshift goes into the pol open reading frame. The pol stop codon is at position 4614. Plasmid ptg8133 from Rhone Merieux contains the FIV gag/pol coding sequences. Part of ptg8133 has been sequenced and the CG at positions 577-578 below is GC in ptg8133, changing the codon from arg to ala);

FIG. 4 shows the ALVAC-nucleotide sequence comprised in the C6 donor plasmid pC6L (SEQ ID NO:3) (Plasmid pC6L contains the C6 insertion sites SmaI (position 409) and EcoRI (position 425));

FIG. 5 shows the predicted nucleotide sequence of the vCP242 insertion (SEQ ID NO:4) (The H6 promotor starts at position 55. The FIV env start codon is at

position 179, and the FIV env stop codon is at position 2749). Positions 1 through 54 and positions 2750 through 2879 flank the H6/FIV env expression cassette);

FIG. 6 shows the predicted nucleotide sequence of I3L promoted FIV gag/protease expression cassette and flanking regions in vCP253 (SEQ ID NO:5) (The I3L promoter begins at position 135. The gag start codon is at position 235 and the protease stop codon is at position 1648);

FIG. 7 shows the predicted nucleotide sequence of the H6 promoted FIV env/I3L promoted FIV gag/protease expression cassette and flanking regions in vCP255 (SEQ ID NO:6) (The H6 promoter starts at position 129, the FIV env start codon is at position 253, and the FIV env stop codon is at position 2823. The I3L promoter starts at position 2830, the FIV gag start codon is at position 2930 and the FIV gag stop codon is at position 4282. The ribosomal frameshift site is near position 4184. The frameshift is -1 in relation to the gag open reading frame. The frameshift goes into the pol open reading frame. The stop codon for the protease gene is at position 4641. Positions 1 through 128 and positions 4642 through 4727 flank the H6 FIV env/I3L FIV gag/protease expression cassette); and

FIG. 8 shows the predicted nucleotide sequence of vCP329 insertion (SEQ ID NO:7) (The H6 promoter starts at position 2146. The coding sequence for FIV 97TM is from position 2022 to position 42. The I3L promoter starts at position 2253. The FIV gag start codon is at position 2353 and the pol stop codon is at position 3766).

DETAILED DESCRIPTION OF THE INVENTION

As discussed above, the invention specifically relates to: vector-based lentivirus, retrovirus, or immunodeficiency virus, e.g., EIAV, FIV, BIV, HIV, or SIV, preferably feline immunodeficiency virus (FIV) recombinants, preferably recombinants containing DNA

encoding an epitope(s) of interest, more preferably Env, Gag, or Pol, or combinations thereof such as Gag and Pol or a portion of Pol, or Env, Gag and Pol or a portion of Pol or Gag and protease, or Env, Gag, and protease, with
5 an attenuated poxvirus such as TROVAC, NYVAC and ALVAC as preferred poxvirus vectors (NYVAC and ALVAC being most preferred, and ALVAC being especially preferred); and, compositions containing the inventive recombinants or expression products therefrom; and to methods for making
10 and using the inventive recombinants, expression products therefrom and compositions including the recombinants and/or expression products.

Thus, in a general way, the invention provides a vector comprising exogenous DNA encoding at least one
15 lentivirus epitope. The epitope can be from a lentivirus other than SIV. More preferably, the epitope is of Gag and Pol or Env, Gag and Pol or Env, Gag and a portion of Pol or Gag and a portion of Pol or Gag-protease, or Env, Gag, and protease; and, most preferably the epitope is
20 Gag and protease or epitope(s) on Gag and protease which elicit a response which is the same as or similar to Gag and protease. And, the vector preferably induces an immune response, more preferably a protective immune response, when administered to a target species (a target
25 species is a host susceptible to the lentivirus; for instance, felines such as domesticated cats and kittens are a target species with respect to FIV).

The methods for making a vector or recombinant can be by or analogous to the methods disclosed in U.S.
30 Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941, 5,338,683, 5,494,807, 5,503,834, 4,722,848, 5,514,375, U.K. Patent GB 2 269 820 B, WO 92/22641, WO 93/03145, WO 94/16716, PCT/US94/06652, U.S. Patent No. 5,833,975.

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35 "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for recombinant gene

- expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.), Smith et al.,
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- 10 Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573,
- EP Patent publication No. 265785, U.S. Patent No. 4,769,331, Roizman, "The function of herpes simplex
- 15 virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA 93:11313-11318,
- 20 October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J.
- 25 Virol. 65, 3068-3075, 1991; Grunhaus et al., 1992, "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, U.S.
- 30 Patent Nos. 6,156,567 and 6,090,393.
- PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993 and McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in
- 35 combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996.

Vaccinia virus and more recently other poxviruses have been used for the insertion and expression of foreign genes. A basic technique of inserting foreign genes into live infectious poxvirus
5 involves recombination between pox DNA sequences flanking a foreign genetic element in a donor plasmid and homologous sequences present in the rescuing poxvirus (Piccini et al., 1987).

Specifically, recombinant poxviruses can be
10 constructed in two steps known in the art and analogous to the methods for creating synthetic recombinants of poxviruses such as the vaccinia virus and avipox virus described in U.S. Patent Nos. 4,769,330, 4,772,848, 4,603,112, 5,110,587, 5,179,993, 5,505,941, and
15 5,494,807,

First, the DNA gene sequence to be inserted into the virus, e.g., an open reading frame from a non-
20 pox source, is placed into a plasmid construct such as an *E. coli* plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted. Separately, the DNA gene sequence to be inserted can be ligated to a promoter. The promoter-gene linkage is
25 positioned in the plasmid construct so that the promoter-gene linkage is flanked on both ends by DNA homologous to a DNA sequence flanking a region of pox DNA; for instance, pox DNA containing a nonessential locus (although an essential locus may also be used). The
30 resulting plasmid construct is then amplified, e.g., by growth within *E. coli* bacteria (Clewell, 1972) and isolated (Clewell et al., 1969; Maniatis et al., 1982). Alternatively, the DNA gene sequence can, without separate ligation to a promoter, merely be placed within
35 the plasmid construct so that the DNA gene sequence is flanked on both ends by DNA homologous to a DNA sequence flanking a region of pox DNA; for instance, a region

downstream from an endogenous promoter such that expression of the gene sequence is under control of the promoter and the promoter and coding portion of the DNA gene sequence are thus adjacent.

5 Second, the isolated plasmid containing the DNA gene sequence to be inserted is transfected into a cell culture, e.g. chick embryo fibroblasts, along with the poxvirus. Recombination between homologous pox DNA in the plasmid and the viral genome respectively gives a
10 poxvirus modified by the presence, e.g., in a nonessential region of its genome, of foreign DNA sequences. The term "foreign" DNA designates exogenous DNA, particularly DNA from a non-pox source, that codes for gene products not ordinarily produced by the genome
15 into which the exogenous DNA is placed.

 However, the foregoing is not meant to limit means for obtaining vectors or recombinants of the present invention, as any means for obtaining a vector or recombinant e.g. a poxvirus-lentivirus, retrovirus,
20 and/or immunodeficiency virus, e.g., feline immunodeficiency virus, recombinant may be used to obtain the present invention.

 Thus, genetic recombination is in general the exchange of homologous sections of DNA between two
25 strands of DNA. In certain viruses RNA may replace DNA. Homologous sections of nucleic acid are sections of nucleic acid (DNA or RNA) which have the same sequence of nucleotide bases.

 Genetic recombination may take place naturally
30 during the replication or manufacture of new viral genomes within the infected host cell. Thus, genetic recombination between viral genes may occur during the viral replication cycle that takes place in a host cell which is co-infected with two or more different viruses
35 or other genetic constructs. A section of DNA from a first genome is used interchangeably in constructing the section of the genome of a second co-infecting virus in

which the DNA is homologous with that of the first viral genome.

However, recombination can also take place between sections of DNA in different genomes that are not perfectly homologous. If one such section is from a first genome homologous with a section of another genome except for the presence within the first section of, for example, a genetic marker or a gene coding for an antigenic determinant inserted into a portion of the homologous DNA, recombination can still take place and the products of that recombination are then detectable by the presence of that genetic marker or gene in the recombinant viral genome. Accordingly, additional strategies have recently been reported for generating recombinant poxviruses such as recombinant vaccinia virus; and, these strategies may be employed in the practice of this invention.

Successful expression of the inserted DNA genetic sequence by the modified infectious virus can occur under two conditions. First, the insertion may be into a nonessential region of the virus in order that the modified virus remain viable, or into an essential region whereby the essential function is not disturbed or the function is not necessary for viability under all conditions. A second condition for expression of inserted DNA is the presence of a promoter in the proper relationship to the inserted DNA. The promoter can be located upstream from the coding portion of the DNA sequence to be expressed.

Vaccinia virus has been used successfully to immunize against smallpox, culminating in the worldwide eradication of smallpox in 1980. In the course of its history, many strains of vaccinia have arisen. These different strains demonstrate varying immunogenicity and are implicated to varying degrees with potential complications, the most serious of which are post-

vaccinial encephalitis and generalized vaccinia (Behbehani, 1983).

With the eradication of smallpox, a new role for vaccinia became important, that of a genetically engineered vector for the expression of foreign genes. Genes encoding a vast number of heterologous antigens have been expressed in vaccinia, often resulting in protective immunity against challenge by the corresponding pathogen (reviewed in Tartaglia et al., 1990a).

The genetic background of the vaccinia vector has been shown to affect the protective efficacy of the expressed foreign immunogen. For example, expression of Epstein Barr Virus (EBV) gp340 in the Wyeth vaccine strain of vaccinia virus did not protect cottontop tamarins against EBV virus induced lymphoma, while expression of the same gene in the WR laboratory strain of vaccinia virus was protective (Morgan et al., 1988).

A fine balance between the efficacy and the safety of a vaccinia virus-based recombinant vaccine candidate is extremely important. The recombinant virus must present the immunogen(s) in a manner that elicits a protective immune response in the vaccinated animal but lacks any significant pathogenic properties. Therefore attenuation of the vector strain would be a highly desirable advance over the current state of technology.

A number of vaccinia genes have been identified which are non-essential for growth of the virus in tissue culture and whose deletion or inactivation reduces virulence in a variety of animal systems.

The gene encoding the vaccinia virus thymidine kinase (TK) has been mapped (Hruby et al., 1982) and sequenced (Hruby et al., 1983; Weir et al., 1983). Inactivation or complete deletion of the thymidine kinase gene does not prevent growth of vaccinia virus in a wide variety of cells in tissue culture. TK⁻ vaccinia virus

is also capable of replication *in vivo* at the site of inoculation in a variety of hosts by a variety of routes.

It has been shown for herpes simplex virus type 2 that intravaginal inoculation of guinea pigs with TK⁻ virus resulted in significantly lower virus titers in the spinal cord than did inoculation with TK⁺ virus (Stanberry et al., 1985). It has been demonstrated that herpesvirus encoded TK activity *in vitro* was not important for virus growth in actively metabolizing cells, but was required for virus growth in quiescent cells (Jamieson et al., 1974).

Attenuation of TK⁻ vaccinia has been shown in mice inoculated by the intracerebral and intraperitoneal routes (Buller et al., 1985). Attenuation was observed both for the WR neurovirulent laboratory strain and for the Wyeth vaccine strain. In mice inoculated by the intradermal route, TK⁻ recombinant vaccinia generated equivalent anti-vaccinia neutralizing antibodies as compared with the parental TK⁺ vaccinia virus, indicating that in this test system the loss of TK function does not significantly decrease immunogenicity of the vaccinia virus vector. Following intranasal inoculation of mice with TK⁻ and TK⁺ recombinant vaccinia virus (WR strain), significantly less dissemination of virus to other locations, including the brain, has been found (Taylor et al., 1991a).

Another enzyme involved with nucleotide metabolism is ribonucleotide reductase. Loss of virally encoded ribonucleotide reductase activity in herpes simplex virus (HSV) by deletion of the gene encoding the large subunit was shown to have no effect on viral growth and DNA synthesis in dividing cells *in vitro*, but severely compromised the ability of the virus to grow on serum starved cells (Goldstein et al., 1988). Using a mouse model for acute HSV infection of the eye and reactivatable latent infection in the trigeminal ganglia, reduced virulence was demonstrated for HSV deleted of the

large subunit of ribonucleotide reductase, compared to the virulence exhibited by wild type HSV (Jacobson et al., 1989).

Both the small (Slabaugh et al., 1988) and
5 large (Schmidt et al., 1988) subunits of ribonucleotide reductase have been identified in vaccinia virus. Insertional inactivation of the large subunit of ribonucleotide reductase in the WR strain of vaccinia virus leads to attenuation of the virus as measured by
10 intracranial inoculation of mice (Child et al., 1990).

The vaccinia virus hemagglutinin gene (HA) has been mapped and sequenced (Shida, 1986). The HA gene of vaccinia virus is nonessential for growth in tissue culture (Ichihashi et al., 1971). Inactivation of the HA
15 gene of vaccinia virus results in reduced neurovirulence in rabbits inoculated by the intracranial route and smaller lesions in rabbits at the site of intradermal inoculation (Shida et al., 1988). The HA locus was used for the insertion of foreign genes in the WR strain
20 (Shida et al., 1987), derivatives of the Lister strain (Shida et al., 1988) and the Copenhagen strain (Guo et al., 1989) of vaccinia virus. Recombinant HA⁻ vaccinia virus expressing foreign genes have been shown to be immunogenic (Guo et al., 1989; Itamura et al., 1990;
25 Shida et al., 1988; Shida et al., 1987) and protective against challenge by the relevant pathogen (Guo et al., 1989; Shida et al., 1987).

Cowpox virus (Brighton red strain) produces red (hemorrhagic) pocks on the chorioallantoic membrane of
30 chicken eggs. Spontaneous deletions within the cowpox genome generate mutants which produce white pocks (Pickup et al., 1984). The hemorrhagic function (u) maps to a 38 kDa protein encoded by an early gene (Pickup et al., 1986). This gene, which has homology to serine protease
35 inhibitors, has been shown to inhibit the host inflammatory response to cowpox virus (Palumbo et al., 1989) and is an inhibitor of blood coagulation.

The u gene is present in WR strain of vaccinia virus (Kotwal et al., 1989b). Mice inoculated with a WR vaccinia virus recombinant in which the u region has been inactivated by insertion of a foreign gene produce higher
5 antibody levels to the foreign gene product compared to mice inoculated with a similar recombinant vaccinia virus in which the u gene is intact (Zhou et al., 1990). The u region is present in a defective nonfunctional form in Copenhagen strain of vaccinia virus (open reading frames
10 B13 and B14 by the terminology reported in Goebel et al., 1990a,b).

Cowpox virus is localized in infected cells in cytoplasmic A type inclusion bodies (ATI) (Kato et al., 1959). The function of ATI is thought to be the
15 protection of cowpox virus virions during dissemination from animal to animal (Bergoin et al., 1971). The ATI region of the cowpox genome encodes a 160 kDa protein which forms the matrix of the ATI bodies (Funahashi et al., 1988; Patel et al., 1987). Vaccinia virus, though
20 containing a homologous region in its genome, generally does not produce ATI. In WR strain of vaccinia, the ATI region of the genome is translated as a 94 kDa protein (Patel et al., 1988). In Copenhagen strain of vaccinia virus, most of the DNA sequences corresponding to the ATI
25 region are deleted, with the remaining 3' end of the region fused with sequences upstream from the ATI region to form open reading frame (ORF) A26L (Goebel et al., 1990a,b).

A variety of spontaneous (Altenburger et al., 1989; Drillien et al., 1981; Lai et al., 1989; Moss et al., 1981; Paez et al., 1985; Panicali et al., 1981) and
30 engineered (Perkus et al., 1991; Perkus et al., 1989; Perkus et al., 1986) deletions have been reported near the left end of the vaccinia virus genome. A WR strain
35 of vaccinia virus with a 10 kb spontaneous deletion (Moss et al., 1981; Panicali et al., 1981) was shown to be attenuated by intracranial inoculation in mice (Buller et

al., 1985). This deletion was later shown to include 17 potential ORFs (Kotwal et al., 1988b). Specific genes within the deleted region include the virokin N1L and a 35 kDa protein (C3L, by the terminology reported in

5 Goebel et al., 1990a,b). Insertional inactivation of N1L reduces virulence by intracranial inoculation for both normal and nude mice (Kotwal et al., 1989a). The 35 kDa protein is secreted like N1L into the medium of vaccinia virus infected cells. The protein contains homology to

10 the family of complement control proteins, particularly the complement 4B binding protein (C4bp) (Kotwal et al., 1988a). Like the cellular C4bp, the vaccinia 35 kDa protein binds the fourth component of complement and inhibits the classical complement cascade (Kotwal et al.,

15 1990). Thus the vaccinia 35 kDa protein appears to be involved in aiding the virus in evading host defense mechanisms.

The left end of the vaccinia genome includes two genes which have been identified as host range genes,

20 K1L (Gillard et al., 1986) and C7L (Perkus et al., 1990). Deletion of both of these genes reduces the ability of vaccinia virus to grow on a variety of human cell lines (Perkus et al., 1990).

To develop a new vaccinia vaccine strain, NYVAC

25 (vP866), the Copenhagen vaccine strain of vaccinia virus was modified by the deletion of six nonessential regions of the genome encoding known or potential virulence factors. The sequential deletions are detailed in U.S. Patents Nos. 5,364,773 and 5,494,807. All designations

30 of vaccinia restriction fragments, open reading frames and nucleotide positions are based on the terminology reported in Goebel et al., 1990a,b.

The deletion loci were also engineered as recipient loci for the insertion of foreign genes.

35 The regions deleted in NYVAC are listed below. Also listed are the abbreviations and open reading frame designations for the deleted regions (Goebel et al.,

1990a,b) and the designation of the vaccinia recombinant (vP) containing all deletions through the deletion specified:

- (1) thymidine kinase gene (TK; J2R) vP410;
- 5 (2) hemorrhagic region (u; B13R + B14R) vP553;
- (3) A type inclusion body region (ATI; A26L) vP618;
- (4) hemagglutinin gene (HA; A56R) vP723;
- (5) host range gene region (C7L - K1L) vP804;
- 10 and (6) large subunit, ribonucleotide reductase (I4L) vP866 (NYVAC).

NYVAC is a genetically engineered vaccinia virus strain that was generated by the specific deletion of eighteen open reading frames encoding gene products associated with virulence and host range. NYVAC is highly attenuated by a number of criteria including i) decreased virulence after intracerebral inoculation in newborn mice, ii) inocuity in genetically (nu⁺/nu⁺) or 20 chemically (cyclophosphamide) immunocompromised mice, iii) failure to cause disseminated infection in immunocompromised mice, iv) lack of significant induration and ulceration on rabbit skin, v) rapid clearance from the site of inoculation, and vi) greatly 25 reduced replication competency on a number of tissue culture cell lines including those of human origin. Nevertheless, NYVAC based vectors induce excellent responses to extrinsic immunogens and provided protective 30 immunity.

Two additional vaccine vector systems involve the use of naturally host-restricted poxviruses, avipox viruses. Both fowlpoxvirus (FPV) and canarypoxvirus (CPV) have been engineered to express foreign gene 35 products. Fowlpox virus (FPV) is the prototypic virus of the Avipox genus of the Poxvirus family. The virus causes an economically important disease of poultry which

has been well controlled since the 1920's by the use of live attenuated vaccines. Replication of the avipox viruses is limited to avian species (Matthews, 1982) and there are no reports in the literature of avipox virus causing a productive infection in any non-avian species including man. This host restriction provides an inherent safety barrier to transmission of the virus to other species and makes use of avipox virus based vaccine vectors in veterinary and human applications an attractive proposition.

FPV has been used advantageously as a vector expressing antigens from poultry pathogens. The hemagglutinin protein of a virulent avian influenza virus was expressed in an FPV recombinant (Taylor et al., 1988a). After inoculation of the recombinant into chickens and turkeys, an immune response was induced which was protective against either a homologous or a heterologous virulent influenza virus challenge (Taylor et al., 1988a). FPV recombinants expressing the surface glycoproteins of Newcastle Disease Virus have also been developed (Taylor et al., 1990; Edbauer et al., 1990).

Despite the host-restriction for replication of FPV and CPV to avian systems, recombinants derived from these viruses were found to express extrinsic proteins in cells of nonavian origin. Further, such recombinant viruses were shown to elicit immunological responses directed towards the foreign gene product and where appropriate were shown to afford protection from challenge against the corresponding pathogen (Tartaglia et al., 1993a; Taylor et al., 1992; 1991b; 1988b).

The ALVAC recombinants can be by the methods detailed in Piccini et al. 1987; Perkus et.al. 1985, e.g., recombination, which is novel and nonobvious with respect the present invention as a novel and nonobvious product results therefrom.

TROVAC refers to an attenuated fowlpox that was a plaque-cloned isolate derived from the FP-1 vaccine

strain of fowlpoxvirus which is licensed for vaccination of 1 day old chicks. TROVAC is a unimolar fowlpox virus species.

ALVAC is an attenuated canarypox virus-based vector that was a plaque-cloned derivative of the licensed canarypox vaccine, Kanapox (Tartaglia et al., 1992). ALVAC has some general properties which are the same as some general properties of Kanapox. ALVAC is a unimolar canarypox virus species.

ALVAC-based recombinant viruses expressing extrinsic immunogens have also been demonstrated efficacious as vaccine vectors (Tartaglia et al., 1993a,b). This avipox vector is restricted to avian species for productive replication. On human cell cultures, canarypox virus replication is aborted early in the viral replication cycle prior to viral DNA synthesis. Nevertheless, when engineered to express extrinsic immunogens, authentic expression and processing is observed *in vitro* in mammalian cells and inoculation into numerous mammalian species induces antibody and cellular immune responses to the extrinsic immunogen and provides protection against challenge with the cognate pathogen (Taylor et al., 1992; Taylor et al., 1990).

Recent Phase I clinical trials in both Europe and the United States of a ALVAC recombinants, e.g., canarypox/rabies glycoprotein recombinant (ALVAC-RG), demonstrated that ALVAC vaccines are safe and well tolerated and, for instance, induced protective levels of rabies virus neutralizing antibody titers (Fries et al., 1996; Pialoux et al., 1995 ; Cadoz et al., 1992). Additionally, peripheral blood mononuclear cells (PBMCs) derived from the ALVAC-RG vaccinates demonstrated significant levels of lymphocyte proliferation when stimulated with purified rabies virus (Fries et al., 1996).

NYVAC, ALVAC and TROVAC have also been recognized as unique among all poxviruses in that the

National Institutes of Health ("NIH") (U.S. Public Health Service), Recombinant DNA Advisory Committee, which issues guidelines for the physical containment of genetic material such as viruses and vectors, i.e., guidelines
5 for safety procedures for the use of such viruses and vectors which are based upon the pathogenicity of the particular virus or vector, granted a reduction in physical containment level: from BSL2 to BSL1. No other poxvirus has a BSL1 physical containment level. Even the
10 Copenhagen strain of vaccinia virus - the common smallpox vaccine - has a higher physical containment level; namely, BSL2. Accordingly, the art has recognized that ALVAC has a lower pathogenicity than other poxvirus.

ALVAC-based recombinant viruses have been shown
15 to stimulate *in vitro* specific CD8⁺ CTLs from human PBMCs (Tartaglia et al., 1993a). Mice immunized with ALVAC recombinants expressing various forms of the HIV-1 envelope glycoprotein generated both primary and memory HIV specific CTL responses which could be recalled by a
20 second inoculation (Tartaglia et al., 1993a; Cox et al., 1993). ALVAC-env recombinants (expressing the HIV-1 envelope glycoprotein) stimulated strong HIV-specific CTL responses from peripheral blood mononuclear cells (PBMC) of HIV-1 infected individuals (Tartaglia et al., 1993a;
25 Cox et al., 1993). Acutely infected autologous PBMC were used as stimulator cells for the remaining PBMC. After 10 days incubation in the absence of exogenous IL-2, the cells were evaluated for CTL activities. ALVAC-env stimulated high levels of anti-HIV activities in mice.
30 These and similar studies (see U.S. Patent No. 5,863,542) show a utility of ALVAC-based recombinants, especially with respect to immunodeficiency viruses. In particular, the highly attenuated character of ALVAC has been demonstrated in both immunocompetent and immuno-
35 compromised animal models in such studies; and, the safety of ALVAC-based recombinants has also been demonstrated.

Thus, in the present invention, the canarypox virus-based ALVAC vector is preferred.

Clearly, based on the attenuation profiles of the ALVAC vectors and its demonstrated ability to elicit
5 both humoral and cellular immunological responses to extrinsic immunogens (Tartaglia et al., 1993a,b; Taylor et al., 1992) such recombinant viruses offer a distinct advantage over previously described vaccinia based recombinant viruses.

10 Perhaps more related to FIV (as felines are involved), an ALVAC-based recombinant virus expressing the FeLV (Subgroup A) env and gag gene products (ALVAC-FL; vCP97) was shown to afford complete protection of cats against an oronasal FeLV challenge exposure
15 (Tartaglia et al., 1993 b). Significantly, protection was afforded in the absence of detectable FeLV-specific serum neutralizing activity prior to challenge.

In certain embodiments of the present invention, Applicants have engineered several ALVAC-FIV
20 recombinants and assessed their ability to afford protection of cats against experimental FIV exposure. In summary, Applicants have demonstrated protection from homologous FIV challenge exposure by vaccination of cats with an ALVAC-FIV Gag-protease recombinant. Recombinants
25 expressing FIV Env alone or in combination with Gag-protease did not afford significant levels of protection. However, vaccination regimens consisting of priming with ALVAC-FIV env/gag-protease and boosting with an adjuvanted inactivated whole cell vaccine preparation
30 provided complete protection, demonstrating utility for the recombinants expressing Env alone or in combination with Gag-protease, despite these recombinants not per se affording significant levels of protection (and further, these recombinants can be used in other aspects of the
35 invention, e.g., to express products which can nonetheless be useful, for instance to obtain useful antibodies, or in kits, tests, assays and the like).

Interestingly, levels of FIV-specific humoral responses measured by ELISA and western blot were not necessarily predictive of protection. Furthermore, Env-specific humoral responses were not associated with the
5 observed protection.

Furthermore, the data herein shows the efficacy of recombinants of the present invention against heterologous FIV challenge in cats, especially in a prime/boost protocol involving an inventive recombinant
10 (e.g., an ALVAC-FIV recombinant) and an ICV.

Moreover, the data herein with respect to FIV and cats is capable of extension to other lentiviruses, retroviruses, and immunodeficiency viruses, e.g., EIAV, FIV, BIV, HIV, or SIV. Thus, knowledge in the art
15 of nucleic acid molecules encoding epitope(s) of interest from these other viruses, e.g., Env, Gag, protease, can be utilized for making and using recombinants expressing epitope(s) of interest analogous to the exemplified FIV data herein. More in particular, using the knowledge in
20 the art of nucleic acid molecules encoding Env, Gag, Pol, or a portion of Pol, such as a portion including protease, accessory functions/proteins, or epitope(s) thereof, for other lentiviruses, retroviruses, and immunodeficiency viruses, e.g., EIAV, FIV, BIV, HIV, or
25 SIV, and using the knowledge in the art of vector systems, the skilled artisan can make vectors or recombinants expressing Env, Gag and Pol or a portion of Pol, or Gag and Pol or a portion of Pol, or Env, Gag and protease, or Gag and protease, with optionally accessory
30 functions/proteins, or expressing epitope(s) thereof, of these other viruses, and can use the vectors or recombinants in an immunization regimen, such as a prime/boost regimen, as herein exemplified with respect to FIV, without any undue experimentation. Accordingly,
35 the invention encompasses vectors or recombinants of lentiviruses, retroviruses and immunodeficiency viruses in addition to FIV (as FIV is a model for other

lentiviruses, retroviruses and immunodeficiency viruses), and methods of making and using those vectors or recombinants.

The expression product generated by inventive
5 vectors or recombinants can also be isolated from infected or transfected cells and used to inoculate hosts in a subunit vaccine configuration (composition, or an antigenic or immunological composition). The proteins generated by the vectors or recombinants and antibodies
10 elicited therefrom can also be used in assays to detect the presence or absence of a lentivirus, retrovirus or immunodeficiency virus, e.g., FIV.

Accordingly, the invention comprehends immunogens or epitope(s) of interest such as lentivirus,
15 retrovirus or immunodeficiency virus immunogen(s) or epitope(s) of interest, e.g., EIAV, FIV, BIV, HIV, or SIV immunogens or epitopes of interest. Indeed, the invention comprehends immunogens or epitopes of interest from lentiviruses, including but not limited to HIV-1,-2,
20 EIAV, BIV. All lentiviruses express functional homologs of the FIV Env, Gag-protease. Techniques for identifying, cloning and utilizing nucleic acid sequences encoding these functional homologs are known in the art and do not require any undue experimentation to practice
25 in the light of this disclosure.

With respect to the state-of-the-art, mention is particularly made of: Gonda et al. (1990). Development of bovine immunodeficiency-like virus as a model of lentivirus disease. Dev. Biol. Stand. 72:97-
30 110; Garvey et al. (1990) Nucleotide sequence and genome organization of biologically active bovine immunodeficiency-like virus. Virology 175:391-409; Gonda et al. (1987). Characterization and molecular cloning of a bovine lentivirus related to human immunodeficiency
35 virus. Nature 330:388-391; Ball et al. (1988). EIAV genomic organization: further characterization by sequencing of purified glycoproteins and cDNA. Virology

165: 601-605; Kawakami et al. (1987) Nucleotide sequence analysis of equine infectious anemia virus proviral DNA. Virology 158: 300-312; Yaniv et al. (1986) Molecular cloning and physical characterization of integrated
5 equine infectious anemia virus:molecular and immunologic evidence of its close relationship to ovine and caprine lentiviruses. Virology 154: 1-8; Stephens et al. (1986). Equine infectious anemia virus gag and pol genes: relatedness to visna and AIDS virus. Science 231:589-594;
10 Chiu et al. (1985). Nucleotide evidence for relationship of AIDS retrovirus to lentiviruses. Nature 317:366-368; as well as a number of reviews in Retrovirus Biology and Human Disease, Gallo, R.C. and Wong-Stall, F. eds. Marcel Dekker, Inc. New York, 1990.

15 Further, DNA encoding such immunogens or epitopes of interest from inventive vectors or recombinants can be administered through immunization using alternate appropriately engineered mammalian expression systems including but not limited to other
20 poxviruses, herpesviruses, adenoviruses, alphavirus-based strategies, and naked or formulated DNA-based immunogens. Techniques for engineering such recombinant subunits are known in the art.

With respect to techniques for these
25 immunization vehicles and state-of-the-art knowledge mention is particularly made of: Hormaeche and Kahn, Perkus and Paoletti, Shiver et al. all in Concepts in Vaccine Development, Kaufman, S.H.E., ed., Walter deGruyter, New York, 1996, and vectors described in
30 Viruses in Human Gene Therapy, Vos, J.-M.H., ed, Chapman and Hall, Carolina Academic Press, New York, 1995, and in Recombinant Vectors in Vaccine Development, Brown, F., ed., Karger, New York, 1994.

The invention still further provides an
35 antigenic, immunogenic, immunological or vaccine composition containing the recombinant virus or expression product thereof, and a acceptable carrier or

diluent. An immunological composition containing the vector or recombinant virus (or an expression product thereof) elicits an immunological response - local or systemic. The response can, but need not be, protective.

- 5 An immunogenic composition containing the vector or recombinant virus (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. An antigenic composition similarly elicits a local or
- 10 systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition", "antigenic composition" and "immunogenic composition" include a "vaccine composition"
- 15 (as the three former terms can be protective compositions). A protective response is understood to be a response, such as a humoral and/or secretory and/or cell-mediated response which confers an immunity, with immunity understood to comprise the ability to resist or
- 20 overcome infection or to overcome infection more easily as compared to a subject not administered the inventive composition, or to better tolerate infection as compared to a subject not administered the inventive composition, e.g., increased resistance to infection.

- 25 As to epitopes of interest, one skilled in the art can determine an epitope or immunodominant region of a peptide or polypeptide and ergo the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as
- 30 from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue experimentation.

- A general method for determining which portions of a protein to use in an immunological composition
- 35 focuses on the size and sequence of the antigen of interest. "In general, large proteins, because they have more potential determinants are better antigens than

small ones. The more foreign an antigen, that is the less similar to self configurations which induce tolerance, the more effective it is in provoking an immune response." Ivan Roitt, Essential Immunology,
5 1988.

As to size: the skilled artisan can maximize the size of the protein encoded by the DNA sequence to be inserted into the mammalian vector (keeping in mind the insertion limitations of the vector). To minimize the
10 DNA inserted while maximizing the size of the protein expressed, the DNA sequence can exclude introns (regions of a gene which are transcribed but which are subsequently excised from the primary RNA transcript).

At a minimum, the DNA sequence can code for a
15 peptide at least 8 or 9 amino acids long. This is the minimum length that a peptide needs to be in order to stimulate a CD4+ T cell response (which recognizes virus infected cells or cancerous cells). A minimum peptide length of 13 to 25 amino acids is useful to stimulate a
20 CD8+ T cell response (which recognizes special antigen presenting cells which have engulfed the pathogen). See Kendrew, *The Encyclopedia of Molecular Biology* (Blackwell Science Ltd 1995). However, as these are minimum lengths, these peptides are likely to generate an
25 immunological response, i.e., an antibody or T cell response; but, for a protective response (as from a vaccine composition), a longer peptide is preferred.

With respect to the sequence, the DNA sequence preferably encodes at least regions of the peptide that
30 generate an antibody response or a T cell response. One method to determine T and B cell epitopes involves epitope mapping. The protein of interest "is fragmented into overlapping peptides with proteolytic enzymes. The individual peptides are then tested for their ability to
35 bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T-cell epitopes since

the T cell recognizes short linear peptides complexed with MHC molecules. The method is less effective for determining B-cell epitopes" since B cell epitopes are often not linear amino acid sequence but rather result
5 from the tertiary structure of the folded three dimensional protein. Janis Kuby, Immunology, pp. 79-80 (1992).

Another method for determining an epitope of interest is to choose the regions of the protein that are
10 hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the regions of the protein which are accessible to the antibody. Janis Kuby, Immunology, p. 81 (1992).

Yet another method for determining an epitope
15 of interest is to perform an X-ray crystallographic analysis of the antigen (full length)-antibody complex. Janis Kuby, Immunology, p. 80 (1992).

Still another method for choosing an epitope of interest which can generate a T cell response is to
20 identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be
25 presented in a MHC complex. The peptide preferably contains appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response. Factors which can be considered are: the HLA type of the patient (vertebrate,
30 animal or human) expected to be immunized, the sequence of the protein, the presence of appropriate anchor motifs and the occurrence of the peptide sequence in other vital cells.

An immune response is generated, in general, as
35 follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex

MHC" located on another cell's surface. There are two classes of MHC complexes - class I and class II, and each class is made up of many different alleles. Different patients have different types of MHC complex alleles; 5 they are said to have a 'different HLA type.'

Class I MHC complexes are found on virtually every cell and present peptides from proteins produced inside the cell. Thus, Class I MHC complexes are useful for killing cells which when infected by viruses or which 10 have become cancerous and as the result of expression of an oncogene. T cells which have a protein called CD4 on their surface, bind to the MHC class I cells and secrete lymphokines. The lymphokines stimulate a response; cells arrive and kill the viral infected cell.

15 Class II MHC complexes are found only on antigen-presenting cells and are used to present peptides from circulating pathogens which have been endocytosed by the antigen-presenting cells. T cells which have a protein called CD8 bind to the MHC class I 20 cells and kill the cell by exocytosis of lytic granules.

Some guidelines in determining whether a protein contains epitopes of interest which will stimulate a T cell response, include: Peptide length - the peptide should be at least 8 or 9 amino acids long to 25 fit into the MHC class I complex and at least 13-25 amino acids long to fit into a class II MCH complex. This length is a minimum for the peptide to bind to the MHC complex. It is preferred for the peptides to be longer than these lengths because cells may cut the expressed 30 peptides. The peptide should contain an appropriate anchor motif which will enable it to bind to the various class I or class II molecules with high enough specificity to generate an immune response (See Bocchia, M. et al., Specific Binding of Leukemia Oncogene Fusion 35 Protein Peptides to HLA Class I Molecules, Blood 85:2680-2684; Englehard, VH, Structure of peptides associated with class I and class II MHC molecules Ann. Rev.

Immunol. 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules. Protein epitopes

5 recognized by T cell receptors are peptides generated by enzymatic degradation of the protein molecule and are presented on the cell surface in association with class I or class II MHC molecules.

Further, the skilled artisan can ascertain an
10 epitope of interest by comparing the protein sequence with sequences listed in the protein data base. Regions of the protein which share little or no homology are better choices for being an epitope of that protein and are therefore useful in a vaccine or immunological
15 composition. Regions which share great homology with widely found sequences present in vital cells should be avoided.

Even further, another method is simply to generate or express portions of a protein of interest,
20 generate monoclonal antibodies to those portions of the protein of interest, and then ascertain whether those antibodies inhibit growth *in vitro* of the pathogen from which the from which the protein was derived. The skilled artisan can use the other guidelines set forth in
25 this disclosure and in the art for generating or expressing portions of a protein of interest for analysis as to whether antibodies thereto inhibit growth *in vitro*.

For example, the skilled artisan can generate portions of a protein of interest by: selecting 8 to 9 or
30 13 to 25 amino acid length portions of the protein, selecting hydrophilic regions, selecting portions shown to bind from X-ray data of the antigen (full length)-antibody complex, selecting regions which differ in sequence from other proteins, selecting potential HLA
35 anchor binding motifs, or any combination of these methods or other methods known in the art.

Epitopes recognized by antibodies are expressed on the surface of a protein. To determine the regions of a protein most likely to stimulate an antibody response one skilled in the art can preferably perform an epitope map, using the general methods described above, or other mapping methods known in the art.

As can be seen from the foregoing, without undue experimentation, from this disclosure and the knowledge in the art, the skilled artisan can ascertain the amino acid and corresponding DNA sequence of a lentivirus epitope of interest for obtaining a T cell, B cell and/or antibody response. In addition, reference is made to Geffer et al., U.S. Patent No. 5,019,384, issued May 28, 1991, and the documents it cites

(Note especially the "Relevant Literature" section of this patent, and column 13 of this patent which discloses that: "A large number of epitopes have been defined for a wide variety of organisms of interest. Of particular interest are those epitopes to which neutralizing antibodies are directed. Disclosures of such epitopes are in many of the references cited in the Relevant Literature section.")

The administration procedure for the inventive vector or recombinant or expression product thereof, compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions can be via a parenteral route (intradermal, intramuscular or subcutaneous). Such an administration enables a systemic immune response. The administration can be via a mucosal route, e.g., oral, nasal, genital, etc. Such an administration enables a local immune response.

More generally, the inventive antigenic, immunological or vaccine compositions or therapeutic compositions (compositions containing the vectors or recombinants of the invention or expression products) can be prepared in accordance with standard techniques well

known to those skilled in the pharmaceutical or veterinary arts. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical and/or veterinary arts taking into

5 consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co-administered or sequentially administered with other compositions of the

10 invention or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions can include purified native antigens or epitopes or antigens or epitopes from the expression by a poxvirus recombinant or another vector system; and are

15 administered taking into account the aforementioned factors.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, genital, e.g., vaginal, etc., administration

20 such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. In such compositions the recombinant may be in admixture

25 with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like.

Antigenic, immunological or vaccine compositions typically can contain an adjuvant and an amount of the recombinant or expression product to elicit

30 the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants are used in research and veterinary applications. Chemically

35 defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff et al., J. Immunol.

147:410-415 (1991)

encapsulation of the protein within a proteoliposome as described by Miller et al., J. Exp. Med. 176:1739-1744 (1992) and

- 5 encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vesicular Systems, Inc., Nashua, NH) can also be used.

The compositions of the invention may be packaged in a single dosage form for immunization by
10 parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or orifice administration, e.g., perlingual (i.e., oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and
15 route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if the vector or recombinant is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host,
20 as well as LD₅₀ and other screening procedures which are known and do not require undue experimentation.

Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5 to 500 µg. The inventive vector or recombinant can be administered in
25 any suitable amount to achieve expression at these dosage levels. The inventive vector or recombinant can be administered to an animal or infected or transfected into cells in an amount of about at least 10^{3.5} pfu; thus, the inventive vector or recombinant is preferably
30 administered to an animal or infected or transfected into cells in at least about 10⁴ pfu to about 10⁶ pfu; however, as shown by the Examples below, animals can be administered at least about 10⁸ pfu such that a more preferred amount for administration can be at least about
35 10⁷ pfu to about 10⁹ pfu. Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The expression product or vector

or recombinant may be lyophilized for resuspension at the time of administration or can be in solution.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly
5 useful in the formulation of a composition having controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nano particles, reported by Kreuter, J., Microcapsules and
10 Nanoparticles in Medicine and Pharmacology, (M. Donbrow, ed.) CRC Press, p. 125-148.

Microencapsulation has been applied to the injection of microencapsulated pharmaceuticals to give a controlled release. A number of factors contribute to
15 the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable
20 release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are polycarbonates, polyesters, polyurethanes, polyorthoesters and polyamides, particularly those that
25 are biodegradable.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,l-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of
30 medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals including peptides and antigens have been formulated into PLGA microcapsules. A body of data has accumulated on the
35 adaption of PLGA for the controlled release of antigen, for example, as reviewed by Eldridge, J.H., et al., Current Topics in Microbiology and Immunology, 1989,

146:59-66. The entrapment of antigens in PLGA microspheres of 1 to 10 microns in diameter has been shown to have a remarkable adjuvant effect when administered orally. The PLGA microencapsulation process
5 uses a phase separation of a water-in-oil emulsion. The compound of interest is prepared as an aqueous solution and the PLGA is dissolved in a suitable organic solvents such as methylene chloride and ethyl acetate. These two immiscible solutions are co-emulsified by high-speed
10 stirring. A non-solvent for the polymer is then added, causing precipitation of the polymer around the aqueous droplets to form embryonic microcapsules. The microcapsules are collected, and stabilized with one of an assortment of agents (polyvinyl alcohol (PVA),
15 gelatin, alginates, polyvinylpyrrolidone (PVP), methyl cellulose) and the solvent removed by either drying in vacuo or solvent extraction.

Thus, solid, including solid-containing-liquid, liquid, and gel (including "gel caps") compositions are
20 envisioned. Additionally, the inventive vector or recombinant, and the expression products therefrom can stimulate an immune or antibody response in animals. From those antibodies, by techniques well-known in the art, monoclonal antibodies can be prepared and,
25 those monoclonal antibodies, can be employed in well known antibody binding assays, diagnostic kits or tests to determine the presence or absence of antigen(s) and therefrom the presence or absence of the natural causative agent of the antigen or, to determine whether
30 an immune response to that agent or to the antigen(s) has simply been stimulated.

Monoclonal antibodies are immunoglobulin produced by hybridoma cells. A monoclonal antibody reacts with a single antigenic determinant and provides
35 greater specificity than a conventional, serum-derived antibody. Furthermore, screening a large number of monoclonal antibodies makes it possible to select an

individual antibody with desired specificity, avidity and isotype. Hybridoma cell lines provide a constant, inexpensive source of chemically identical antibodies and preparations of such antibodies can be easily

- 5 standardized. Methods for producing monoclonal antibodies are well known to those of ordinary skill in the art, e.g., Koprowski, H. et al., U.S. Pat. No. 4,196,265, issued Apr. 1, 1989,

- 10 Uses of monoclonal antibodies are known. One such use is in diagnostic methods, e.g., David, G. and Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983,

- Monoclonal antibodies have also been used to
15 recover materials by immunoadsorption chromatography, e.g. Milstein, C., 1980, Scientific American 243:66, 70,

- Furthermore, the inventive vector or recombinant or expression products therefrom can be used
20 to stimulate a response in cells *in vitro* or *ex vivo* for subsequent reinfusion into a patient. If the patient is seronegative, the reinfusion is to stimulate an immune response, e.g., an immunological or antigenic response such as active immunization. In a seropositive patient,
25 the reinfusion is to stimulate or boost the immune system against the lentivirus, retrovirus, or immunodeficiency virus, e.g., FIV.

- Accordingly, the inventive vector or recombinant has several utilities: In antigenic,
30 immunological or vaccine compositions such as for administration to seronegative animals or humans (or patients, as veterinarians like to call animals, with "patients" including humans as well). In therapeutic compositions in seropositive animals or humans in need of
35 therapy to stimulate or boost the immune system against the lentivirus, retrovirus, or immunodeficiency virus, e.g., feline immunodeficiency virus. *In vitro* to produce

antigens or immunogens or epitopes of interest, which can be further used in antigenic, immunological or vaccine compositions or in therapeutic compositions. To generate antibodies (either by direct administration or by
5 administration of an expression product of the inventive vectors or recombinants) which can be further used: in diagnosis, tests or kits to ascertain the presence or absence of antigens or epitopes in a sample such as sera, for instance, to ascertain the presence or absence of the
10 lentivirus, retrovirus, or immunodeficiency virus, e.g., feline immunodeficiency virus, in a sample such as sera or, to determine whether an immune response has elicited to the lentivirus, retrovirus, or immunodeficiency virus, e.g., FIV, or, to particular antigen(s) or epitope(s);
15 or, in immunoadsorption chromatography. To generate DNA for use as hybridization probes or to prepare PCR primers or for DNA immunization. And, the inventive vectors or recombinants, expression products therefrom, and immunogens, antigens, and epitopes from the inventive
20 vectors or recombinants can be used to generate stimulated cells which can be further used (reinfused) to stimulate an immune response (antigenic, or immunological response; or active immunization) or, to boost or stimulate the immune system (for instance, of an
25 immunocompromised or seropositive animal or human). Other utilities also exist for embodiments of the invention.

A better understanding of the present invention and of its many advantages will be had from the following
30 examples, given by way of illustration.

EXAMPLES

DNA Cloning and Synthesis. Plasmids were constructed, screened and grown by standard procedures (Maniatis et al., 1982; Perkus et al., 1985; Piccini et
35 al., 1987). Restriction endonucleases were obtained from Bethesda Research Laboratories, Gaithersburg, MD, New England Biolabs, Beverly, MA; and Boehringer Mannheim

Biochemicals, Indianapolis, IN. Klenow fragment of *E. coli* polymerase was obtained from Boehringer Mannheim Biochemicals. BAL-31 exonuclease and phage T4 DNA ligase were obtained from New England Biolabs. The reagents
5 were used as specified by the various suppliers.

Synthetic oligodeoxyribonucleotides were prepared on a Biosearch 8750 or Applied Biosystems 380B DNA synthesizer as previously described (Perkus et al., 1989). DNA sequencing was performed by the dideoxy-chain
10 termination method (Sanger et al., 1977) using SequenaseTM (Tabor et al., 1987) as previously described (Guo et al., 1989). DNA amplification by polymerase chain reaction (PCR) for sequence verification (Engelke et al., 1988) was performed using custom synthesized oligonucleotide
15 primers and GeneAmpTM DNA amplification Reagent Kit (Perkin Elmer Cetus, Norwalk, CT) in an automated Perkin Elmer Cetus DNA Thermal Cycler. Excess DNA sequences were deleted from plasmids by restriction endonuclease digestion followed by limited digestion by BAL-31
20 exonuclease and mutagenesis (Mandecki, 1986) using synthetic oligonucleotides.

Cells, Virus, and Transfection. The origins and conditions of cultivation of the Copenhagen strain of vaccinia virus has been previously described (Guo et al.,
25 1989). Generation of recombinant virus by recombination, in situ hybridization of nitrocellulose filters and screening for B-galactosidase activity are as previously described (Piccini et al., 1987).

The origins and conditions of cultivation of
30 the Copenhagen strain of vaccinia virus and NYVAC and ALVAC has been previously described (Guo et al., 1989; Tartaglia et al., 1992, U.S. Patents Nos. 5,364,773 and 5,494,807). Generation of recombinant virus by recombination, in situ hybridization of nitrocellulose
35 filters and screening for B-galactosidase activity are as previously described (Panicali et al., 1982; Perkus et al., 1989).

The parental canarypox virus (Rentschler strain) is a vaccinal strain for canaries. The ALVAC vaccine strain, as discussed above, was obtained from a wild type isolate and attenuated through more than 200 serial passages on chick embryo fibroblasts. A master viral seed was subjected to four successive plaque purifications under agar and one plaque clone was amplified through five additional passages after which the stock virus was used as the parental virus in in vitro recombination tests. The plaque purified canarypox isolate is designated ALVAC.

The strain of fowlpox virus (FPV) designated FP-1 has been described previously (Taylor et al., 1988a). It is an attenuated vaccine strain useful in vaccination of day old chickens. The parental virus strain Duvette was obtained in France as a fowlpox scab from a chicken. The virus was attenuated by approximately 50 serial passages in chicken embryonated eggs followed by 25 passages on chicken embryo fibroblast cells. The virus was subjected to four successive plaque purifications. One plaque isolate was further amplified in primary CEF cells and a stock virus, designated as TROVAC, established.

NYVAC, ALVAC and TROVAC viral vectors and their derivatives were propagated as described previously (Piccini et al., 1987; Taylor et al., 1988a,b, U.S. Patents Nos. 5,364,773 and 5,494,807). Vero cells and chick embryo fibroblasts (CEF) were propagated as described previously (Taylor et al., 1988a,b).

30 **EXAMPLE 1 - Construction of ALVAC-FIV env**

The feline immunodeficiency virus (FIV) env coding sequence in plasmid ptg6184 and FIV nucleotide sequences were obtained from Rhone Merieux (Lyon, France). The cDNA clone was derived from the Villefranche strain of FIV. The FIV env nucleotide sequence is shown in Figure 2 (SEQ ID NO:1).

The FIV env coding sequence was placed under control of the modified early/late vaccinia virus H6 promoter (Perkus, et al., 1989). The FIV env coding sequence contains two T₅NT sequence motifs which may
 5 provide for premature early transcription termination (Yuen and Moss, 1987). The T₅NT sequences were modified, without altering the predicted amino acid coding sequence, by replacement with a PCR-derived fragment. TTTTAT between positions 2059 and 2065 in Figure 2 was
 10 changed to TTCTTAT; TTTTCT between positions 2110 and 2116 was changed to TTCTTCT.

Two overlapping PCR fragments were derived from the ptg6184 template, yielding a fragment with altered T₅NT sequences. A 585bp PCR fragment was generated using
 15 oligonucleotide primers MM040 (SEQ ID NO:9) (5'-AAATTCTTATATACAGCTTTCGCTATGCAAGAATTAGGATGTAATCAAAATCAATTC TTCT GCAAAATCCCTCCTGGGT-3') and MM042 (SEQ ID NO:10) (5'-CCCATCGAGTGCGGCTAC-3'). MM040 primes toward the 3'-most sequences of the env coding sequence (from position 2056,
 20 Figure 2). MM042 primes from the env 3'-most sequences toward the 5'-most sequences. A second PCR primed with MM041 (SEQ ID NO:11) (5'GCAGAAGAATTGATTTTGATTACATCCTAATTCTT GCATAGCGAAAGCTGTATATAAGAATTTTCCATAGCTTC-3') and MM043
 25 (SEQ ID NO:12) (5'AAGTTCTGGCAACCCATC-3') generated a 187bp fragment. MM041 primes from position 2118 toward the 5'-most-sequences of env and MM043 primes toward the 3'-most sequences of the env coding sequence from position 1931 (Figure 2). The two PCR products were
 30 pooled, primed with MM043 and MM042, and digested with ScaI at FIV coding sequence position 2020 in Figure 2 and EcoRI 3' of the env coding sequence. The resultant 564bp ScaI-EcoRI PCR fragment contains the 3'-most sequences of the FIV env coding sequence with the altered T₅NT motifs.
 35 Plasmid ptg6184 was digested with EcoRI and partially digested with ScaI. This ptg6184 derived fragment with the 3' FIV env deleted from ScaI (Figure 2

position 2020) through EcoRI 3' of the env coding sequence was ligated to the 564bp ScaI-EcoRI PCR-derived fragment (above). The resultant plasmid pMM120 contains the FIV env with altered T₅NT motifs. The nucleotide

5 sequence was confirmed using standard procedures (Goebel et al., 1990a). The 2.6kbp pMM120 PstI-EcoRI fragment, containing the FIV env coding sequence, was inserted between the pBS-SK (Stratagene, La Jolla, California) PstI and EcoRI sites generating pMM122.

10 The modified early/late vaccinia virus H6 promoter (Perkus et al., 1989) was added to pMM122 by overlapping the H6 translation initiation codon with the FIV env translation initiation codon. A fragment

15 containing the H6 promoted 5'-most sequences of the env coding sequence was generated by PCR using primers MM037 (SEQ ID NO:13) (5' ATCATCCTGCAGAAGCTTCCCGGGTTCTTTATTCTATACTT-3'), MM038 (SEQ ID NO:14) (5'-CTGCAAATCCTTCTGCCATTACGATACAAACTTAAC-3'), MM065 (SEQ ID NO:15) (5'-

20 CGTTAAGTTTGTATCGTAATGGCAGAAGGATTTGCAGCC-3'), and MM036 (SEQ ID NO:16) (5'-CCTCTTGAATTTTCGTTCC-3'). pMM108, containing H6 promoter sequences, was used as template for PCR with MM037 and MM038 creating a 166bp fragment containing the H6 promoter and the 5'-most bp of the FIV

25 env coding sequence. pMM122 was used as template for PCR with MM065 and MM036 to generate a 235bp fragment with the 3' H6 promoter and 5' env end. The two PCR products were pooled, primed with MM036 and MM037, and the resultant fragment, containing the H6 promoter fused to

30 the 5' most bp of the FIV env coding sequence, was digested with PstI and KpnI generating a 266bp fragment. pMM122 was digested with PstI and partially digested with KpnI to remove the 5'-most

sequences of the FIV env coding region and the 266bp

35 PstI-KpnI PCR-derived fragment described above was inserted. The resultant plasmid pMM125 contains the FIV

env juxtaposed 3' to the vaccinia H6 promoter in pBS-SK (Stratagene, La Jolla, California).

Sequence analysis of pMM125 demonstrated correct PCR construction of the H6 promoted FIV env 5'-most sequences, but frameshift mutations were observed 3' of the PCR insertion. The frameshifts were not observed in pMM122. All pMM125 clones contained frameshifts. These frameshifts were probably a result of the recently described instability of env sequences in high copy number plasmids (Wang and Mullins, 1995). Separate pMM125 clones had different frameshifts. An H6 promoted FIV env, without frameshifts, was constructed in the following manner.

Briefly, this is a summary of the following detailed description of the construction of an H6 promoted FIV env without frameshifts. The H6 promoted 5'-most sequences of the FIV env coding sequence, which did not contain frameshifts, from one pMM125 clone was ligated to the remaining unframeshifted 3'-most bp of FIV env end from another pMM125 clone. The first fragment was from the SmaI site 5' of the H6 promoter through the AflII site in the FIV env coding sequence (Figure 2, position 1707). The second fragment was from the same AflII site to the SmaI site 3' of the env coding sequence. The ligation product was digested with SmaI, liberating three fragments. One fragment contained two 5'-most sequences and another fragment contained two 3'-most sequences. The third SmaI digestion product containing the H6 promoted FIV env expression cassette was isolated and inserted into a C6 vector, generating pRW945. To eliminate the possibility of frameshifts, pRW945 was not amplified in bacteria. Details of pRW945 construction follow.

One pMM125 clone, pMM125#11, had the correct sequence from SmaI 5' of the H6 promoter through the AflII site at position 1707 (Figure 2); another pMM125 clone, pMM125#10, had the correct sequence from the AflII

site at position 1707 through SmaI 3' of the env coding sequence. The 1.8kbp pMM125#11 SmaI-AflII fragment, containing the H6 promoted 5'-most env sequences, was ligated to the 0.9kbp pMM125#10 SmaI-partial AflII fragment containing the 3' portion of env. The ligation product was SmaI digested and the 2.7kbp fragment was inserted into the C6 vector pMM117, yielding pRW945.

The C6 insertion plasmid, pMM117, was constructed in the following manner. A 3kbp ALVAC HindIII clone was sequenced and an open reading frame was defined. A PCR-derived fragment was used for replacement of the open reading frame with restriction sites for DNA insertions. The PCR-derived fragment was generated with primers C6A1 (SEQ ID NO:17) (5'-ATCATCGAGCTCGCGGCCGCCTATCAAAAGTCTTAATGAGTT-3'), C6B1 (SEQ ID NO:18) (5'-GAATTCCTCGAGCTGCAGCCCGGGTTTTTATAGCTAATTAGTCATTTTTTCGTAAGTAAGTATTTTTATTAA-3'), C6C1 (SEQ ID NO:19) (5'-CCCGGGCTGCAGCTCGAGGAATTCTTTTTATTGATTAAGTCAAATGAGTATATAAATTGA AAAAGTAA-3') and C6D1 (SEQ ID NO:20) (5'-GATGATGGTACCTTCATAAATACAAGTTTGATTAACTTAAGTTG-3'). ALVAC was used as template for PCR using oligonucleotides C6A1 and C6B1 generating a 380bp fragment. A second PCR reaction used ALVAC template and primers C6C1 and C6D1 to generate a 1155bp fragment. The PCR reaction products were pooled and primed for a final PCR with C6A1 and C6D1 yielding a 1613bp fragment. The final PCR product was digested with SacI and KpnI for insertion between the SacI and KpnI sites of pBS-SK (Stratagene, La Jolla, California). The resultant C6 insertion plasmid was designated pC6L. The C6 insertion plasmid pMM117 was constructed by adding the sequence GGGGGATCCTTAATTAATTAGTTATTAGACAAGGTGAAAACGAAACTATTTGTAGCTTAATTAATTAGCTGCAGGAATTC (SEQ ID NO:21) between the pC6L (Fig. 4; SEQ ID NO:3) SmaI and EcoRI sites.

The above described plasmid pRW945 contains the H6 promoted FIV env coding sequence, with altered T₅NT motifs, in a C6 insertion plasmid. pRW945 was used in *in vitro* recombination experiments with ALVAC as the rescuing virus to derive recombinant vCP242. Figure 5 shows the predicted nucleotide sequence of the insertion in vCP242 (SEQ ID NO:4).

EXAMPLE 2 - Construction of Recombinant ALVAC
Expressing FIV gag, and pro

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The feline immunodeficiency virus (FIV) gag and pol coding sequences in plasmid ptg8133 and FIV env coding sequence in plasmid ptg6184 were obtained from Rhone Merieux (Lyon, France). The cDNA clones were derived from the Villefranche strain of FIV. Figure 3 (SEQ ID NO:2) contains the nucleotide sequence for the FIV gag and pol coding regions obtained from Rhone Merieux.

The FIV gag sequences encoding core antigens, followed by the pol sequences encoding a protease, reverse transcriptase and integrase, were placed under control of the early/intermediate vaccinia I3L promoter (Schmitt, J. and Stunnenberg, H., 1988; Vos, J. and Stunnenberg, H., 1988). The I3L promoter corresponds to positions 65073 through 64971 in Goebel et al., 1990 a,b. The gag and pol coding sequences were engineered in a single transcription unit. The Gag and Pol open reading frames (ORFS) differ and translation of the Pol ORF results via a ribosomal frameshift mechanism (Morikawa and Bishop, 1992) as it does normally in FIV-infected cells.

PCR-derived fragments were used for construction of the I3L promoted FIV gag/pol cassette. The PCR-derived fragments were also used to alter a T₅NT sequence motif which may provide for premature early transcription termination (Yuen and Moss, 1987). TTTTAT between positions 467 and 473 (Figure 3) was changed to

TTTTCAT, without altering the predicted amino acid coding sequence. Manipulations to construct the I3L promoted FIV gag/pol expression cassette were performed in the following manner.

5 PCR was performed with ptg8133, containing the FIV gag/pol coding sequences, as template and MM027 (SEQ ID NO:22) (5'-CAAAAATGGTGTCCATTTTTCATGGAAAAGGCAAGAGAAGGAC-3') and MM028 (SEQ ID NO:23) (5'-CTGCTGCAGTAAAATAGG-3') as primers to generate a 245bp fragment. MM027 primed
10 from position 452 (Fig. 3) toward the 3'-most sequences containing a nucleotide change in the T₅NT sequence motif. MM028 primes from position 697 downstream of a HindIII site toward the gag 5'-most sequences. The 245bp PCR-derived fragment contains the FIV gag coding sequence
15 from position 452 to position 697 with an altered T₅NT motif.

A second PCR using ptg8133 as template and primers MM029 (SEQ ID NO:24) (5'-CTTCTCTTGCCTTTTCCATGAAAATGGACACCATTTT
20 TGGGTC-3') and MM030 (SEQ ID NO:25) (5'-CAATTATTTAGGTTTAATCATGGGGAATGGACAGGGGC-3') generated a 508bp fragment. MM029 primes from position 490 (Fig. 3) toward the 5'-most sequences of the gag coding sequence and alters the T₅NT sequence motif. MM030 contains the
25 3'-most sequence of the I3L promoter and primes from the gag initiation codon toward the 3'-most sequences of the gag coding sequence. The 508bp PCR-derived fragment contains the 3'-most I3L promoter and the FIV gag coding sequence with an altered T₅NT motif through position 490.

30 Plasmid template pMM100, containing the I3L promoter sequences, was primed with MM031 (SEQ ID NO:26) (5'-CGCCCCTGTCCATTCCCATGATTAAACCTAAATAATTGTAC-3') and MM032 (SEQ ID NO:27) (5'-ATCATCGTCGACATCGATACATCATGCAGTGGTTAAAC-3') to generate a
35 137bp PCR-derived fragment. The MM031 5'-most sequences contains the 5'-most bp of gag followed by a sequence which primes at the I3L promoter 3'-most sequences toward

the I3L promoter 5'-most sequences. MM032 has SalI and ClaI sites followed by a sequence which primes from the I3L promoter 5'-most sequences toward the 3'-most sequences. The 137bp PCR-derived fragment contains the

5 I3L promoted FIV gag 5'-most 20bp.

The three PCR products were pooled and primed for PCR with MM032 and MM028. The resultant 814bp fragment was digested with HindIII and SalI, generating a 726bp fragment containing the I3L promoted FIV gag 5'-

10 most sequences with an altered T₅NT motif.

ptg8133 was digested with SacI and SalI, to remove the 7.2kbp plasmid sequences, and the 4.7kbp fragment was isolated and partially digested with HindIII. The 4kbp ptg8133 SacI-HindIII partial digestion

15 product, containing the FIV gag coding sequence from position 615 through the FIV pol coding sequence, was isolated.

SacI-SalI digested pBS-SK (Stratagene, La Jolla, California) was ligated with the 726bp HindIII-SalI PCR-derived fragment (above) and the 4kbp ptg8133 SacI-HindIII fragment. The resultant plasmid pMM116 contains the I3L promoted FIV gag/pol expression cassette in pBS-SK.

20

The 4.7kbp pMM116 Asp718-Ecl136II fragment containing I3L promoted FIV gag/pol coding regions was treated with Klenow, in the presence of 20mM dNTPs, and inserted into SmaI digested pMM117 to produce pMM121. pMM117 is the C6 insertion plasmid described above.

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The 1.4kbp pMM121 EcoRI fragment, containing the I3L promoted FIV gag/pol 5'-most region, was inserted into the pBS-SK (Stratagene, La Jolla, California) EcoRI site generating pMM123. A PCR-derived fragment was used to remove the coding sequences corresponding to the carboxy-end of Pol to achieve Gag-protease expression

30 only. The PCR-derived fragment introduced a termination codon following the protease coding sequence at position 1709 (Fig. 3). Manipulations to construct the I3L

35

promoted FIV gag and protease coding sequences were performed in the following manner.

Template pMM121, containing the I3L promoted FIV gag/pol coding sequences, was primed with MM063 (SEQ ID NO:28) (5'-CAGGACATCTAGCAAGAC-3') and MM064 (SEQ ID NO:29) (5'-GATGATCCCGGGATAAAAATTATTGAGCCATTACTAACCT-3') to generate a 580bp PCR-derived fragment. MM063 primes from position 1148 (Fig. 3) toward the 3'-most sequences. MM064 primes from position 1709 toward the 5'-most sequences. The 580bp PCR-derived fragment, containing the FIV protease coding sequence with a stop codon at position 1709 (Fig. 3), was digested with EcoRI and SmaI yielding a 475bp fragment.

pMM123 was linearized at the SmaI site 3' of the FIV insertion, followed by partial EcoRI digestion. The 475bp SmaI-EcoRI PCR-derived fragment (above) was inserted into the pMM123 SmaI-EcoRI partial digestion product, with the EcoRI site digested at figure 3 position 1246. The resultant plasmid pMM127 contains the FIV gag and protease coding sequences, followed by a stop codon, in pBS-SK (Stratagene, La Jolla, California). The nucleotide sequence of the PCR-derived fragment in pMM127 was confirmed using standard procedures (Goebel et al., 1990a). A single bp deletion 3' of the FIV protease coding sequence was observed. MM064 was designed to add TTTTAT after the FIV protease stop codon. One T in the TTTTAT sequence after the stop codon is missing from pMM127, resulting in the sequence TTTTAT.

The 1.8kbp pMM127 BamHI-SmaI fragment, containing the I3L promoted FIV gag and protease coding sequences, was inserted into SmaI-BamHI partially digested pMM117. The C6 insertion plasmid pMM117 is described above. The BamHI partial digestion was used to digest the BamHI site next to the SmaI site in pMM117. The resultant plasmid, containing the I3L promoted FIV gag and protease coding sequences in a C6 insertion plasmid, was designated pMM129. Plasmid pMM129 was used

in *in vitro* recombination experiments with ALVAC as the rescuing virus to derive recombinant vCP253. Figure 6 (SEQ ID NO:5) shows the predicted nucleotide sequence of the I3L promoted FIV gag/protease expression cassette and flanking regions in vCP253.

EXAMPLE 3 - Construction of Recombinant ALVAC
Expressing FIV env, gag, and pro

Plasmid pMM125, containing the H6 promoted FIV env with frameshift mutations, is described above. A deliberate insertion, containing a frameshift, into the FIV env coding sequence allowed stable maintenance of the remainder of the H6 promoted FIV env construct in bacteria. After bacterial amplification the insertion was removed. Manipulations to construct the H6 promoted FIV env coding sequence, with a deliberate frameshift insertion, were performed in the following manner.

pMM125#11 (described above) was modified by insertion of a PCR-derived fragment which repaired the spontaneous frameshift and introduced a deliberate frameshift flanked by BstEII sites. The BstEII insertion is at position 1920 (Fig. 2). The insertion introduces a stop codon followed by a frameshift, NotI and HindIII sites. There are no BstEII sites in pMM125. The PCR-derived fragment also changes the A at position 1920 in figure 2 to C. The A to C change does not alter the predicted amino acid coding sequence, but the change does introduce a BstEII site. pMM125#10 has a spontaneous frameshift at position 1604 (Figure 2). pMM125#10 was used as template for PCR with oligonucleotide primers RW542 (SEQ ID NO:30) (5'-TATGAATTGTAATTGTAC-3') and RW545 (SEQ ID NO:31) (5'-GTAGCATAAGGTTACCGCGGCCGCTAAGCTTAGGTTACCATCCCTATAGCAGTA-3') to generate a 326bp fragment containing the BstEII insertion. RW542 primes from position 1632 toward the FIV env 3'-most sequences; RW545 primes from position 1919 toward the FIV env 5'-most sequences (Figure 2). pMM125#10 was used as template for PCR with RW544 (SEQ ID

NO:32) (5'-
 GTAGCATAAGGTAACCTAAGCTTAGCGGCCGCGGTAAACCCAATACCACCAAGTTCTG
 GC-3') and T3 (SEQ ID NO:33) (5'-ATTAACCCTCACTAAAG-3')
 generating a 791bp fragment containing the BstEII
 5 insertion and the FIV env coding sequence 3'-most
 sequences. RW544 primes from position 1914 toward the
env 3'-most sequences. T3 primes in the pBS-SK plasmid,
 downstream of the FIV env stop codon, toward the FIV env
 5'-most sequences. The two PCR products were pooled,
 10 primed with RW542 and T3, and the resultant 1.1Kbp
 product was digested with EcoRI and partially digested
 with AflIII generating a 876bp fragment which was inserted
 into the following pMM125#11 vector. The PCR-derived
 fragment and pMM125#11 vector were digested with AflIII at
 15 position 1709 (Fig. 2). pMM125#11 was digested with
EcoRI and EcoRI to remove the FIV env coding sequence 3'-
 most sequences which contained a spontaneous frameshift.
 The 876bp EcoRI-AflIII PCR-derived fragment was inserted
 into the pMM125#11 EcoRI-AflIII vector. The resultant
 20 plasmid pMM134 contains the FIV env coding sequence
 juxtaposed 3' to the H6 promoter in pBS-SK (Stratagene,
 La Jolla, California). pMM134 also contains a deliberate
 frameshift mutation inserted between two BstEII sites.
 The entire H6 promoted FIV env sequence in pMM134,
 25 including the BstEII insertion, was confirmed. As
 expected, the BstEII insertion allowed stable maintenance
 of the remainder of the H6 promoted FIV env construct.

Once the H6 promoted FIV env coding sequence
 from pMM134 is cloned into a poxvirus insertion plasmid,
 30 the BstEII insertion should be removed to allow
 expression of the full length FIV env coding sequence.
 The BstEII insertion is removed by BstEII digestion,
 followed by a dilute ligation reaction favoring
 intramolecular ligation. The intramolecular ligation
 35 product would contain the H6 promoted FIV env, without
 the BstEII insertion. After the BstEII insertion is
 removed, the H6 promoted FIV env coding sequence is not

expected to be stably maintained in bacteria and the plasmid was not amplified in bacteria. After ligation, the plasmid was digested with NotI. Ligation products containing the BstEII insertion would be digested at the

5 NotI site within the BstEII insertion. NotI digestion within the BstEII insertion would prevent the ability of the plasmid to generate a viable recombinant poxvirus. Full length FIV env, without the BstEII insertion, would not be cleaved by NotI digestion; FIV env coding

10 sequences would remain intact.

EXAMPLE 4 - Construction of The H6 Promoted FIV env Coding Sequence In C6 With The I3L Promoted FIV gag And Protease Coding Sequences

15 Construction of pMM134 containing the H6 promoted FIV env coding sequence with a BstEII insertion has been described above. The 2.7kbp H6 promoted FIV env SmaI fragment from pMM134, with the BstEII insertion, was cloned into the following pMM129 insertion plasmid.

20 pMM129, containing the I3L promoted FIV gag and protease coding sequences in C6, has been described above. The pMM129 SalI site 5' of the I3L promoter was blunt ended with Klenow in the presence of 20mM dNTPs. pMM138 was constructed by insertion of the 2.7kbp pMM134

25 SmaI fragment containing the H6 promoted FIV env coding sequence, with the BstEII insertion, into the pMM129 blunt ended SalI site. The H6 promoted FIV env coding sequence, in pMM138, is 5' of the I3L promoted FIV gag and protease coding sequences; the FIV coding sequences

30 are transcribed in the same direction.

The two BstEII sites in pMM138 surround the insertion containing a frameshift. Digestion of pMM138 with BstEII, to remove the insertion, was followed by ligation. The resultant plasmid pMM146 was not amplified

35 in bacteria. pMM146 was designed for NotI digestion before *in vitro* recombination experiments; NotI digestion served two purposes. First, any plasmid unintentionally

containing the BstEII insertion would be digested with NotI within the insertion and the donor plasmid would be prevented from generating a viable ALVAC recombinant. Second, NotI linearizes pMM146 within the pBS-SK backbone for efficient generation of the intended ALVAC recombinant. pMM146 was digested with NotI prior to use in *in vitro* recombination experiments with ALVAC as the rescuing virus to derive recombinant vCP255. Figure 7 (SEQ ID NO:6) shows the predicted nucleotide sequence of the H6 promoted FIV env/I3L promoted FIV gag/protease expression cassette and flanking regions in vCP255.

**EXAMPLE 5 - Construction of Recombinant ALVAC
Expressing FIV 97TM, gag, and pro**

The FIV envelope glycoprotein is composed of two cleavage products, gp97 and gp40. The FIV env coding sequence was modified, by replacing gp40 with the transmembrane anchor domain from the FIV env coding sequences, in the following FIV 97TM construct. FIV 97TM, containing gp97 followed by the transmembrane anchor domain, was constructed in the following manner.

A PCR-derived fragment PCR-FIV1 (242bp) was synthesized using pMM125#10 (containing the previously described FIV env with the correct sequence from AflII site to 3'-most sequences) as a template, and oligonucleotides MW196 (SEQ ID NO:34) (5'-ACTTGCCATCGTCATGGGGG-3') and MW195A (SEQ ID NO:35) (5'-GATACCTCCCAATAGTCCCCTTTTCCTTCTAGGTTTATATTC-3') as primers. PCR-derived fragment PCR-FIV2 (193bp) was synthesized using pMM125#10 as a template, and oligonucleotides MW194A (SEQ ID NO:36) (5'-GAATATAAACCTAGAAGGAAAAGGGGACTATTGGGAGGTATC-3') and MW197 (SEQ ID NO:37) (5'-ATCATCGAATTCATAAAAATCATTTCTTCTCCTTCTACTTC-3') as primers. PCR-derived fragment PCR-FIV3 (393bp) was synthesized using PCR-derived fragments PCR-FIV1 and PCR-FIV2 as templates, and oligonucleotides MW196 and MW197 as

primers. A complete AflIII/EcoRI digest of PCR-FIV3 was performed, and the 284bp fragment was isolated. This fragment was ligated into the 4.8kb AflIII/EcoRI fragment of pMM125#11 (containing the previously described FIV env with the correct sequence from 5'-most sequences to the EcoRI site described above). The resultant plasmid, pMAW103, contains H6 promoted FIV 97TM.

A PstI site was added upstream of the H6 promoter in the following manner. PCR-derived fragment PCR-FIV4 (359bp) was synthesized using pMAW103 as a template, and oligonucleotides MW209 (SEQ ID NO: 8) (5'-ATCATCAAGCTTCTGCAGTTCTTTATTCTA TACTTA-3') and MM036 (SEQ ID NO:16) (5'-CCTCTTGAATTTTCGTTCC-3') as primers. A complete HindIII/NruI digest of PCR-FIV4 was performed, and the 110bp fragment was inserted into the 5.0kb HindIII/NruI fragment of pMAW103, yielding plasmid pMAW103A. The 2126bp pMAW103A PstI fragment containing the H6 promoted FIV 97TM was inserted into the PstI site of pMM117 (described above), yielding plasmid pMAW104.

The 2852bp pMAW104 BamHI partial digestion product, containing H6 promoted FIV 97TM, was inserted into BamHI digested pMM129 (I3L promoted FIV gag and pro in C6 described above). The resultant plasmid pMAW105 contains H6 promoted FIV 97TM and I3L promoted FIV gag and pro in C6. Plasmid pMAW105 was used in in vitro recombination experiments with ALVAC as the rescuing virus to derive recombinant vCP329. Figure 8 (SEQ ID NO:7) shows the predicted nucleotide sequence of the insertion to make vCP329.

EXAMPLE 6 - ALVAC FIV Recombinant Expression Analysis

Expression of the appropriate FIV-specific gene products encoded by the ALVAC recombinants vCP242, vCP253, vCP255, and vCP329 was demonstrated in various analyses. Expression analyses were performed using either appropriate monoclonal antibodies or serum derived from FIV seropositive cats. Either reagent worked

equally well in confirming expression in ALVAC-FIV-infected cells. Accordingly, without undue experimentation, from seropositive individuals, monoclonals can be derived for confirming expression.

5 vCP242 FIV Env expression was demonstrated by ELISA (described below). vCP242 was positive for surface expression in an immunofluorescence assay by FACS with an FIV Env specific monoclonal antibody (obtained from Rhone-Merieux, Lyon, France). vCP242 was positive by
10 immunoprecipitation using polyclonal serum from FIV infected cats and two different monoclonal antibodies (described below). Thus, without undue experimentation, monoclonals from seropositive individuals can be derived for confirming expression.

15 vCP253 was positive for internal expression of Gag by FACS. vCP253 was positive by immunoprecipitation for expression of the mature Gag p24. A dominant Gag precursor was detected at 37kDa; additional signals, representing Gag cleavage products, were obtained at
20 49kDa, 40kDa, and 32kDa.

vCP255 surface expression for Env was positive by FACS with an Env-specific monoclonal antibody (described below). vCP255 internal expression of Gag was demonstrated with a Gag-specific monoclonal antibody by
25 FACS. vCP255 was assayed by immunoprecipitation with monoclonal antibodies to each gene product: Gag was positive with signals at approximately 49kDa, 40kDa, 37kDa, and 24kDa; FIV Env expression was positive with signals at 130kDa and 90kDa.

30 vCP329 expression of 97TM and gag were detected by immunoprecipitation with pooled serum from FIV infected cats.

FACS ANALYSIS: vCP255 contains the feline immunodeficiency virus (FIV) env, gag and protease coding
35 sequences in locus C6. pMM146 was used in *in vitro* recombination experiments with ALVAC as the rescuing virus to derive recombinant vCP255. vCP255 FIV-specific

gene product expression was assayed on a fluorescence activated cell sorter (FACS). The FIV Env protein product was assayed on the surface of vCP255 infected cells. The FIV p24 product was assayed for using
5 internal expression analyses. The antisera used for FACS analysis were:

- 1) monoclonal anti-FIV env: 128F10 EP110592 from Rhone Merieux (1:200 dilution)
- 2) monoclonal anti-FIV p24: pool 125A3, 314B5
10 EP072092 from Rhone Merieux (1:100 dilution)
- 3) monoclonal anti-rabies G: 24-3F-10 021387 from C. Trimarchi, Griffin Laboratories, New York State Health Department (1:200 dilution)
- 4) polyclonal goat anti-mouse IgG coupled to
15 fluorescein isothiocyanate (FITC) from Boehringer Mannheim, catalogue number 605240, lot number 24064 (1:100 dilution)

FACS ANALYSIS OF EXPRESSION ON CELL SURFACE: 1

10^7 HeLa-S3 cells (ATCC #CCL2.2) were infected with 5×10^7 PFU of vCP255 in minimum essential medium (S-MEM: Gibco #380-2380AJ) supplemented with 10% fetal bovine sera, 20mM Glutamine and 0.5% penicillin-streptomycin. The infected cells were incubated at 37°C for 30 minutes with occasional agitation. The cells were washed with
25 10mls S-MEM. After each wash the cells were pelleted at 1000 RPM for 5 minutes in a Beckman GPKR centrifuge. The infected cell pellet was resuspended in 1ml S-MEM, transferred to a 5ml Sarstadt tube and slowly rotated at 37°C overnight.

30 After overnight incubation, 100 μ l aliquots of the infected cells were added to 5ml polypropylene tubes. The cells were washed with 3mls of PBS-CMF (137mM NaCl, 2.7mM KCl, 1.5mM KH₂PO₄, and 8mM Na₂HPO₄; pH 7.4) which included 0.2% NaN₃ and 0.2 % bovine serum albumen (BSA).
35 The cells were pelleted and the supernatant was discarded. Specific antibody was added to one tube and

nonspecific antibody (anti-rabies G) was added to a second tube in the following manner.

100 μ l of antibody (previously preadsorbed with HeLa Cells) diluted in PBS-CMF supplemented with 0.2% NaN₃ and 0.2% BSA was added to each cell pellet, and incubated at 4°C for 30 minutes. The cells were washed and pelleted twice in 3mls of PBS-CMF containing 0.2% NaN₃ and 0.2% BSA. 100 μ l of secondary FITC coupled antibody (previously preadsorbed with HeLa Cells) diluted 1:50 in PBS-CMF containing 0.2% NaN₃ and 0.2% BSA was added and incubated at 4°C, in the dark, for 30 minutes. The cells were washed and pelleted twice in 3mls of PBS-CMF containing 0.2% NaN₃ and 0.2% BSA. The cell pellets were resuspended in 1ml PBS-CMF, containing 0.2% NaN₃ and 0.2% BSA, transferred to 5ml polystyrene tubes and assayed on the FACS.

FACSCAN ANALYSIS OF INTERNAL EXPRESSION:

1 x 10⁷ HeLa-S3 cells (ATCC# CCL2.2) were infected with 5 x 10⁷ PFU of vCP255 in minimum essential medium (S-MEM: Gibco #380-2380AJ) supplemented with 10% fetal bovine serum, 20mM Glutamine and 0.5% penicillin-streptomycin. The infected cells were incubated at 37°C for 30 minutes with occasional agitation. The cells were washed with 10mls S-MEM. After each wash the cells were pelleted at 1000 RPM for 5 minutes in a Beckman GPKR centrifuge. The infected cell pellet was resuspended in 1ml S-MEM, transferred to a 5ml Sarstadt tube and slowly rotated at 37°C overnight.

After overnight incubation, 100 μ l aliquots of the infected cells were added to 5ml polypropylene tubes. The cells were washed with 3mls PBS-CMF which contained 0.2% NaN₃. 100 μ l of 4% paraformaldehyde (Polysciences Inc. #00380) pH 7.4 in PBS-CMF containing 0.2% NaN₃ was added to the cell pellet and incubated on ice for 10 minutes. Specific antibody was added to one tube and nonspecific antibody (anti-rabies G) was added to a second tube in the following manner.

The paraformaldehyde treated cells were washed with 3mls PBS-CMF containing 0.2% NaN_3 . Following the wash, 100 μl PBS-CMF containing 0.2% NaN_3 , 1% saponin (SIGMA S-7900) and 20% heat inactivated newborn calf serum (Gibco #200-6010AJ) was added. The cells were incubated on ice for 30 minutes and washed with 3mls PBS-CMF which contained 0.2% NaN_3 .

100 μl of antibody (previously preadsorbed with HeLa Cells) diluted in PBS-CMF supplemented with 0.1% saponin and 20% heat inactivated newborn calf serum was added to each cell pellet, and incubated at 4°C for 30 minutes. The cells were washed and pelleted twice in 3mls of PBS-CMF containing 0.2% NaN_3 and 0.1% saponin.

100 μl of secondary antibody coupled to FITC (previously preadsorbed with HeLa Cells) diluted 1:50 in PBS-CMF containing 0.2% NaN_3 and 0.1% saponin and 20% heat inactivated newborn calf serum was added and incubated at 4°C, in the dark, for 30 minutes. The cells were washed and pelleted twice in 3mls of PBS-CMF containing 0.2% NaN_3 and 0.1% saponin. The cell pellets were resuspended in 1ml PBS-CMF-containing 0.2% NaN_3 , transferred to 5ml polystyrene tubes and assayed on the FACS.

VCP255 FACS ANALYSIS: Antisera/HeLa suspensions were assayed on a Becton Dickinson model FC FACSscan flow cytometer. Data was analyzed on Lysis II Software (Becton Dickinson, UK). The antisera/HeLa suspensions were excited with a 488 nm argon laser, and FITC emission spectra was identified using FL-1 channel detectors. Ungated data was collected on 10,000 cells.

Fluorescence emission spectra, obtained by FACS analysis of ALVAC infected HeLa cells, demonstrated background levels of rabies G and FIV-specific gene products. Background levels of the rabies G glycoprotein were obtained by FACS analysis of vCP255 infected HeLa cells.

The fluorescence emission spectra of vCP255 infected HeLa cells, probed with FIV specific monoclonal

antibodies, demonstrated expression of the FIV-specific gene products. The FIV p24 coding sequence product was detected internally from vCP255 infected HeLa cells. The FIV Env product was detected on the surface of vCP255
5 infected HeLa cells.

IMMUNOPRECIPITATION ANALYSIS: CEF or VERO cells were infected at an m.o.i. of 10 pfu/cell with ALVAC (the parental virus), vCP242, vCP253, vCP255 or vCP329. Following an hour adsorption period, the
10 inoculum was removed and the cells were overlaid with 2mls of modified Eagle's medium (minus cysteine and methionine) containing 2% dialyzed fetal bovine serum and [³⁵S]-TRANSlabeled (New England Nuclear, Boston, MA; 30uCi/ml). The lysates were harvested 18-24 hrs post-
15 infection by addition of 1ml 3X buffer A (450mM NaCl, 3% NP-40, 30mM Tris (pH7.4), 3mMEDTA, 0.03% Na-azide, and 0.6 mg/ml PMSF) and analyzed for expression of FIV env and gag gene products. The above described polyclonal cat antisera or FIV-specific monoclonal antibodies were
20 used for immunoprecipitation analysis in the following manner.

Lysates were incubated overnight at 4°C with FIV-specific antisera-protein A-sepharoseTM complexes. The samples were washed 4X with 1X buffer A and 2X with a
25 LiCl₂/urea buffer (200mM LiCl, 2M urea, and 10mM Tris pH8.0). Precipitated proteins were dissociated from the immune complexes by addition of 2X Laemmli buffer (124mM Tris (pH6.8), 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and boiling for 5 minutes. Proteins
30 were fractionated on 10% SDS-polyacrylamide gels, fixed in methanol, and treated with 1M Na-salicylate for fluorography. Proteins of the appropriate size were precipitated from the lysates derived from cells infected with the ALVAC-FIV recombinants, but were not
35 precipitated from uninfected or ALVAC infected cells. The results indicated appropriate expression of the FIV gene products by the ALVAC-FIV recombinants.

ELISA ANALYSIS: Primary chick embryo fibroblast (CEF) cells were infected with vCP219, vCP242, or ALVAC. The infected cells were analyzed with the following FIV-specific monoclonal antibodies (Rhone
5 Merieux, Lyon, France).

FIV gag: 0126B4 (anti-P15)

314B5 (anti-P24)

125A3 (anti-p24)

FIV env: 128F10

10 117E5

115G8

SERUM SAMPLES TESTED BY ELISA:

1. Serum from FIV-infected cats:

Received from Rhone Merieux.

15 Cats #34 and #103

2. Normal cat serum: Cat #1229 (Select Labs, Athens, GA).

3. Rabbit serum obtained from immunization with vCP65 (ALVAC-RG):

20 Rabbit A039: prebleed
week 14

Infected cell lysates were prepared in the following manner. Roller bottles of CEF cells were infected with ALVAC, vCP219, or vCP242 at an MOI of 5 PFU
25 per cell in serum-free medium. Each roller bottle was harvested at 20 hours post infection, when the cells were completely round but not detached. Harvest consisted of pouring off the medium, washing once with PBS, and scraping the cells in 3 ml of PBS supplemented with
30 aprotinin (3.6 T.I.U; Sigma #A-6279). The harvested cells were sonicated for four minutes on ice, and then centrifuged for 10 min at 1000xg. Supernates were recovered and the protein concentration was approximately 7 mg/ml for each preparation.

35 A kinetic ELISA was performed in the following manner. Serum samples (above) were assayed by a sandwich kinetic ELISA for the detection of FIV env and gag gene

products. Microtiter plates were coated with the pooled monoclonal antibodies, listed above, specific for either FIV env or FIV gag, at 2 or 5 $\mu\text{g}/\text{well}$. Infected cell lysates were applied at 0.2, 1, or 5 $\mu\text{g}/\text{well}$, for capture
5 by the monoclonals. Each serum sample was assayed in triplicate at a dilution of 1:100. Antibody was detected with a 1:200 dilution of horse radish peroxidase(HRP)-conjugated anti-cat serum (Jackson Immuno Research cat# 102-035-003) or HRP-conjugated anti-rabbit serum (DAKO,
10 cat# P217), followed by HRP substrate, o-phenylenediamine dihydrochloride(OPD). The optical densities at A_{450} were read for 15 min and rates for each sample were calculated as MOD per minute.

Results from these ELISAs clearly demonstrated
15 that FIV Env and Gag expression were detected with serum from FIV infected cats, but not normal cat serum (data not shown). Env was demonstrated with plates prepared using Env-specific MAb and lysates derived from cells infected with vCP242, and not with lysates from ALVAC or
20 vCP219 infected cells. Similarly, Gag was demonstrated with plates prepared using Gag-specific MAb and lysates from cells infected with vCP219, and not ALVAC or vCP242 infected cells.

TABLE 1. DETECTION OF FIV ENV EXPRESSION BY KINETIC ELISA

CAT SERUM: ^b	ALVAC lysate ^a		env lysate		gag lysate		
	NCS	FIV	NCS		FIV	NCS	FIV
lysate conc (μ g/well)	KELISA (mOD/min)						
0.2	1.3	5.2	1.2		5.1	1.2	3.5
1	1.7	5.0	1.4		11.3	1.7	4.9
5	2.2	5.4	1.6		22.0	2.0	5.0
RABBIT SERUM: ^c	PB	Wk 14	PB		Wk 14	PB	Wk 14
0.2	1.3	4.8	0.9		2.3	0.9	2.8
1	1.0	5.0	0.7		4.1	0.8	3.0
5	1.1	6.4	0.9		3.0	1.0	4.6

a Cell lysates from CEF cells infected with ALVAC, ALVAC-FIV env, or ALVAC-FIV gag were applied at 0.2, 1, or 5 μ g/well to wells previously coated with 2 μ g/well of pooled FIV env-specific MAb.

b Cat sera: normal cat (NCS), FIV-infected cats (FIV).

c Rabbit sera: prebleed (PB) and week 14 serum from rabbits inoculated with vCP-65 (NYVAC-RG).

TABLE 2 DETECTION OF FIV GAG-SPECIFIC ANTIBODIES BY KINETIC ELISA

	ALVAC	lysate ^a	env lysate	gag	lysate	
CAT SERUM ^b	NCS	FIV	NCS	FIV	NCS	FIV
lysate conc (μ g/ml)	KELISA (mOD/min)					
0.2	2.7	6.7	1.5	3.9	1.4	10.4
1	2.3	6.2	1.8	3.6	1.3	30.7
5	2.7	6.5	1.9	4.3	1.7	32.2
RABBIT SERUM ^c	PB	wk 14	PB	wk 14	PB	wk 14
0.2	1.3	5.1	1.0	4.0	1.3	4.4
1	1.1	6.0	1.2	4.0	1.2	4.1
5	1.2	6.6	1.0	4.3	1.0	4.4

a Cell lysates from CEP cells infected with ALVAC, ALVAC-FIV env, or ALVAC-FIV gag were applied at 0.2, 1, or 5 μ g/well to wells previously coated with 2 μ g/well of pooled FIV gag-specific MAb.

b Cat sera: normal cat (NCS), FIV-infected cats (FIV).

c Rabbit sera: prebleed (PB) and week 14 serum from rabbits inoculated with vCP-65 (NYVAC-RG).

EXAMPLE 7 - Efficacy of ALVAC-FIV Recombinants In Cats

Grouping And Immunization: A total of 36 SPF animals purchased from Liberty (Waverly, NY), age 12 weeks, were divided into seven groups as follows:

5	GroupsA	GroupB	GroupC	GroupD	GroupE	GroupF	GroupG
	QH4F	QH5F	QQ1F	QQ2M	QH2M	QH3M	QC5F
10	PY1M	PY3M	QA5F	PY5F	PY2M	PY4M	QG4F
	OO1F	QS4F	QU2F	QO2F	QA4F	QA6F	QE4M
	QC1M	QC3M	QX3M	QX4M	QC4M		
	QU1M	QG3F	QI1M	QI2M	QG5F		
	QL2F	QE2M	QL3F	QL4M	QE3F		

Immunizations were administered as follows:

15 <u>Group A (6 cats)</u>	Immunization
(Days)	
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Primary immunization:	ALVAC-env (vCP242)	Day 0
Secondary immunization:	ALVAC-env	Day 28
Tertiary immunization:	ALVAC-env	Day 56

20

Group B (6 cats)

Primary immunization: ALVAC-env , gag/pro
(vCP255) Day 0

25	Secondary immunization: ALVAC-env, gag/pro	Day 28
	Tertiary immunization: ALVAC-env, gag/pro	Day 56

Group C (6 cats)

30	Primary immunization: ALVAC-gag/pro (vCP253)	Day 0
	Secondary immunization: ALVAC-gag/pro	Day 28
	Tertiary immunization: ALVAC-gag/pro	Day 56

Group D (6 cats)

- Primary immunization: ALVAC-97TM gag/pro
(vCP329) Day 0
- 5 Secondary immunization: ALVAC-97TM gag/pro Day 28
- Tertiary immunization: ALVAC-97TM gag/pro Day 56

Group E (6 cats/control)

- 10 Primary immunization: ALVAC (CPpp) Day 0
- Secondary immunization: ALVAC Day 28
- Tertiary immunization: ALVAC Day 56

Group F (3 cats/boost)

- 15 Primary immunization: ALVAC-env , gag/pro
(vCP255) Day 0
- Secondary immunization: ALVAC-env, gag/pro Day 28
- Boost: Inactivated FIV cell vaccine (ICV) Day 56

20

Group G (3 cats/control)

- Primary immunization: ALVAC Day 0
- Secondary immunization: ALVAC Day 28
- 25 Boost: Inactivated FIV cell vaccine Day 56

- All cats received 1×10^8 PFU of the respective ALVAC recombinant in 1 ml sterile PBS via the intramuscular route. The ICV boost consisted of 2.5×10^7
- 30 fixed allogenic FIV-Petaluma infected feline T-cells (Fl-4 cell line), mixed with 250 μ g muramyl dipeptide (Hosie et al., 1995). The ICV boost was given subcutaneously.

- Challenge: All cats were challenged via an intraperitoneal (IP) administration 4 weeks following
- 35 final immunization with 50 CID_{50} of FIV-Petaluma (cell free supernatant derived from PBMC cultures infected with FIV Petaloma strain).

The following assays were performed to determine the FIV-specific virological status of the challenged animals. This provided a direct measurement of the protective efficacy of the ALVAC-based FIV vaccine candidates.

1) Virus Isolation: Detection of Infectious FIV by RT Assay. Peripheral blood mononuclear cells (PBMCs), bone marrow (BM) cells, and lymph node (LN) cells were collected upon challenge for virus isolation (Yamamoto et al., 1991, 1993; Okada et al., 1994). Virus isolation was performed by monitoring reverse transcriptase (RT) activity of culture supernatants. Isolated cells were cultured in the presence of IL-2 for 4 weeks. One-ml aliquots by standard procedures for Mg^{++} -dependent RT activity (specific for lentiviruses).

2) FIV-specific PCR. Proviral sequence detection was performed on DNA extracted from PBMC, BM, and LN cells. As a means of increasing the sensitivity, four consensus primer sets were used to amplify either env- or gag-specific coding regions, respectively (Yamamoto et al., 1991; Okada et al., 1994).

Following the initial PCR amplification, 1/25th of the product was re-amplified with the nested primer pair.

The results of the virological assays for samples pre- and post-challenge are presented in Tables 3 and 4. None of the cats demonstrated FIV viremia prior to challenge assessed either by RT determination or by the FIV-specific PCR analysis (Table 3). By 8 weeks post-challenge 4 of the 6 cats immunized with three doses of the ALVAC parental virus developed a persistent FIV-specific viremia (Table 3). Infection of these cats was also demonstrable by virus isolation and PCR in tissue samples taken post-challenge and by apparent FIV-specific seroconversion post-challenge (Table 4 and 5). No clear indications of infection were observed in the other two cats (QA4 and QE3) in the control group. Further, in

comparison to this control group, no significant differences in efficacy were observed in groups of cats receiving three inoculations (10^8 pfu/dose) of ALVAC-FIV env (vCP242), ALVAC-FIV env/gag-pr (vCP255), or ALVAC-FIV 97TMG (vCP329).

Significantly, three administrations of ALVAC-FIV gag-pr(vCP253) afforded complete protection against FIV challenge exposure. Protection from infection was clearly evident in six of six cats throughout the 29 week post-challenge observation period by virus isolation and FIV-specific PCR in the periphery and lymphoid tissue (Table 3 and 4). Further, these cats also did not seroconvert relative to FIV seroreactivity by Western blot or ELISA (Table 4 and 5). To further substantiate the efficacy of vCP253, cells (PBMCs, lymph node, and bone marrow) from two animals in this group were transferred to SPF kittens. These cats have thus far tested negative by virus isolation (RT and PCR) and FIV-specific Western blot, whereas an SPF-cats receiving similar cells from an infected control cat (Py2) clearly was positive for infection by these criteria.

Collectively, these results show that the Gag-pr is sufficient to protect against a lentivirus challenge exposure. As shown in Table 6, these results are indeed statistically significant. The results also show that the presence of Env may actually interfere with the establishment of a protective immune response. Further, the data for the experimental arm where cats received vCP255 (2x) followed by ICV immunogen illustrated that any impairment of Env can be overcome by such a prime/boost regimen (Table 3 and 4). Clearly the priming activity contributed by vCP255 was useful for protection, since the cats in the group receiving 2 administrations of ALVAC parental virus followed by ICV were readily infected upon challenge exposure (Table 3 and 4).

In short, this data provides for the first time protection against FIV infection in cats using a subunit immunogen, including only the FIV Gag-pr. In fact, the presence of Env may have reduced efficacy.

- 5 The importance of such data is also apparent in general for lentivirus vaccine development. Protection using solely the Gag-pr provides several important elements to vaccine and diagnostic design. First, one can readily employ existing Env-based assays to
- 10 discriminate vaccinated versus infected individuals. Secondly, the Gag-pr appears less variable than the Env species between lentivirus isolates and thus may serve for provision of cross-protective responses.

TABLE 3

Virus isolation (Reverse Transcriptase assay and PCR on PBMC)

Vaccine	Cat no.	Pre		Post challenge							
				4 weeks		8 week		12 weeks		17 weeks	
				RT	PCR	RT	PCT	RT	PCT	RT	PCT
ALVAC- Env	QH4	-	-	-	-	-	-	-	-	-	-
	PY1	-	-	+	+	+	+	+	+	+	+
	QO1	-	-	+	+	+	+	T	T	+	+
	QC1	-	-	-	-	-	-	-	-	-	-
	QU1	-	-	-	-	-	-	-	-	-	-
	QL2	-	-	+	+	+	+	+	+	+	+
ALVAC- Eng, gag/prot	QH5	-	-	-	-	-	-	-	-	-	-
	PY3	-	-	-	-	-	-	-	-	-	-
	QS4	-	-	+	+	+	+	+	+	+	+
	QC3	-	-	-	-	-	-	-	-	-	-
	QG3	-	-	-	-	-	-	-	-	-	-
	QE2	-	-	-	-	-	-	+	+	-	+
ALVAC- gag/prot	QQ1	-	-	-	-	-	-	-	-	-	-
	QA5	-	-	-	-	-	-	-	-	-	-
	QU2	-	-	-	-	-	-	-	-	-	-
	QX3	-	-	-	-	-	-	-	-	-	-
	QI1	-	-	-	-	-	-	-	-	-	-
	QL3	-	-	-	-	-	-	-	-	-	-
ALVAC- 97TM of gag/prot	QQ2	-	-	-	-	-	-	-	-	-	-
	PY5	-	-	-	-	-	-	-	-	-	-
	QO2	-	-	+	+	+	+	T*	T*	-	-
	QX4	-	-	+	+	+	+	+	+	-	-
	QI2	-	-	-	+	+	+	+	+	+	+
	QL4	-	-	-	-	-	-	-	-	-	-
ALVAC- control	QH2	-	-	+	+	+	+	T*	-	-	-
	PY2	-	-	-	-	+	+	+	+	+	+
	QA4	-	-	-	-	-	-	-	-	-	-
	QC4	-	-	-	-	+	+	+	+	+	+
	QG5	-	-	+	+	+	+	T*	-	-	-
	QE3	-	-	-	-	-	-	-	-	-	-
ALVAC- env, gag, prot & ICV	QH3	-	-	-	-	-	-	-	-	-	-
	PY4	-	-	-	-	-	-	-	-	-	-
	QA6	-	-	-	-	-	-	-	-	-	-
ALVAC- control & ICV	QC5	-	-	+	+	+	+	+	+	T*	-
	QG4	-	-	+	+	+	+	+	+	T*	-
	QE4	-	-	-	-	+	+	+	+	+	+

*T: Animal was euthanized.

ND: Not Determined

TABLE 4

FINAL VIRUS ISOLATION ON PBMC and TISSUE SAMPLES

Vaccine	Cat no.	TISSUE SAMPLE										
		PBL RT	PCR	LN RT	PCR	BM RT	PCR	THY RT	PCR	WB	Elisa	Tissues taken at x weeks post-chall.
ALVAC-Env	QH4	-	"+"	-	"+"	-	"+"	-	-	-	-	27
	PY1	-	-	-	+	-	-	+	+	+	+	24
	QO1	+	+	+	+	+	+	ND	ND	+	+	10
	QC1	-	-	-	-	-	-	ND	ND	-	-	28
	QU1	-	-	-	-	-	-	ND	ND	-	-	28
	QL2	-	-	+	+	+	+	-	-	+	+	24
ALVAC-Env, gag/prot	QH5	-	-	-	-	-	-	-	-	ND	-	28
	PY3	-	-	-	-	-	-	ND	ND	-	-	27
	QS4	-	-	+	+	-	+	-	-	+	+	24
	QC3	-	-	-	-	-	-	-	-	-	-	28
	QG3	-	-	-	-	-	-	-	-	-	-	28
	QE2	-	"+"	-	+	-	+	-	"+"	+	+	28
ALVAC-gag/prot	QQ1	-	-	-	-	-	-	ND	ND	-	-	29
	QA5	-	-	-	-	-	-	ND	ND	-	-	29
	QU2	-	-	-	-	-	-	ND	ND	-	-	29
	QX3	-	-	-	-	-	-	ND	ND	-	-	29
	QI1	-	-	-	-	-	-	ND	ND	-	-	29
	QL3	-	-	-	-	-	-	ND	ND	-	-	29
ALVAC-97TH OF gag/prot	QQ2	-	-	-	+	-	-	-	-	-	-	27
	PY5	-	-	-	-	-	-	-	-	-	-	28
	QO2	+	+	+	+	+	+	ND	ND	+	+	10
	QX4	-	-	-	-	-	-	-	-	+	-/+	25
	QI2	-	-	-	-	-	-	-	-	+	-	25
	QL4	-	-	-	-	-	-	-	-	ND	-	27
ALVAC-control	QH2	+	+	+	+	+	+	ND	ND	+	-/+	10
	PY2	+	+	-	-	-	+	ND	ND	+	-	39
	QA4	-	-	-	-	-	-	-	-	-	-	28
	QC4	+	+	+	+	+	+	+	+	+	+	26
	QG5	-	+	+	+	-	+	ND	ND	+	+	10
	QE3	-	-	-	-	-	-	-	-	-	-	28
ALVAC-Env/gag/prot & ICV	QH3	-	-	-	-	-	-	ND	ND	+	-	36
	PY4	-	-	-	-	-	-	ND	ND	+	-	36
	QA6	-	-	-	-	-	-	ND	ND	+	-	36
ALVAC-control & ICV	QC5	+	+	+	+	-	-	ND	ND	+	+	10
	QG4	-	+	+	+	-	+	ND	ND	+	+	10
	QE4	+	+	ND	ND	+	+	ND	ND	+	+	39

*T: Animal was euthanized.

ND: Not Determined

"+": Show only very faintly positive by PCR.

NOTE Westernblot: serum dilution 1:100

ELISA: serum dilution 1:200, Transmembrane peptide used: QELGCNQNQFFCK1

EXAMPLE 8 - ALVAC-FIV Recombinants induce protective Immunity
Against Multiple Subtype FIV Challenge In
Cats

5 MATERIALS AND METHODS

Animals: Specific pathogen free (SPF), were purchased from Liberty Research, Inc.

Vaccine preparation: Canarypoxvirus (ALVAC)-FIV recombinants were generated as described above (vCP255).

- 10 The ALVAC vCP255 vaccine was prepared from a serum free lysate of infected CEF. ALVAC vCP255 immunizations were given at 1×10^8 PFU intramuscularly. The inactivated cell vaccine (ICV) consisted of 2×10^8 paraformaldehyde inactivated Fl-4 cells (a feline lymphoid cell line
- 15 chronically infected with FIV Petaluma) mixed with $250 \mu\text{g}$ SAF/MDP adjuvant (Hoise et al., 1995) and was given subcutaneously.

- Grouping and immunization protocol: The challenge study involved 6 cats; the ALVAC-
- 20 env,gag/pro/ICV immunized group (#PY4, #QH3, #QA6) which received the FIV Petaluma challenge described in Example 7 and a control group of three age matched SPF cats (#EJ2, #DH3, #GU5) which had received no immunizations prior to the FIV Bangston challenge. (See Tables 5).

- 25 Challenge: The second challenge inoculum consisted of 75 ID₅₀ cell free FIV Bangston (subtype B) and was given 8 months after the initial FIV Petaluma challenge (See Example 7).

- Immunogenicity monitoring: The induction of
- 30 FIV specific antibody responses were determined by Western blotting (immunoblot). Viral neutralizing antibody responses (VNA) were determined using previously described assays (Yamamoto et al., 1991).

- Viral infectivity monitoring: Viral infection
- 35 was monitored by several methods. This included assessment of viral reverse transcriptase activity in PBMC, bone marrow and lymph node cells taken at various times post-challenge by previously described methods (Yamamoto et al., 1991). In addition, pro-viral DNA
- 40 (latent infection) was monitored by polymerase chain

reaction (PCR) using FIV-env primers on DNA extracted from PBMC, bone marrow and lymph node cells upon culturing for RT activity Yamamoto et al., 1991; Okada et al., 1994). Further, FIV infection was determined by monitoring and comparing the character of FIV-specific humoral responses and viral neutralizing (VN) antibody responses in serum taken before and after challenge.

RESULTS AND DISCUSSION

The immunogenicity and the protective efficacy of ALVAC prime/boost protocols was evaluated against experimental challenge with a distinctly heterologous FIV isolate (Bangston strain). First, the protective efficacy of immunizing with ALVAC-*env,gag/pol* alone was compared to priming with ALVAC-*env,gag/pol* followed by boosting with inactivated FIV-infected cell vaccine (ICV). All cats received a total of three immunizations and were challenged 4 weeks after the final immunization with cell free 50 ID₅₀ of FIV Petaluma (See Example 7). The FIV Petaluma isolate, like the FIV Villefranche isolate used to generate the ALVAC-FIV recombinant vaccine, is classified as a subtype A virus and differs from FIV Villefranche 3% in the Env and 1% in the Gag amino-acid coding region.

It was then evaluated whether the ALVAC-*env,gag/pro/ICV* immunized cats (#QA6, #QH3, #PY4) which resisted the FIV Petaluma challenge described in Example 7, could be protected from a second challenge with a distinctly heterologous FIV isolate of another subtype. The second challenge consisted of 75 ID₅₀ cell free FIV Bangston (PBMC derived) and was given at eight months after the initial challenge without any intervening booster. FIV Bangston is a subtype B isolate and differs from FIV Petaluma (subtype A) by 21% in the envelope glycoprotein amino acid coding region. Three age matched SPF cats (#EJ2, #GU5, #DH3) served as control cats for the FIV Bangston challenge. As presented in Table 7, all control cats became readily infected upon challenge. In contrast, ALVAC-*env,gag/pro/ICV* immunized cats #QH3 and #QA6 remained virus negative as determined by virus isolation (RT) and PCR of peripheral blood up to three

months post-challenge. Cat #PY4 remained virus negative as determined by virus isolation (RT) of peripheral blood but tested positive by PCR at three months post-challenge. Nucleotide sequence analysis of the PCR product revealed FIV Bangston specific sequences. Thus ALVAC-env,gag/pro/ICV immunized cats were partially protected from a heterologous subtype FIV challenge. It is clear that these cats demonstrated, at least, a delay in infection as all control cats became viremic by 6 weeks post-challenge and only one of three ALVAC-env,gag/pol/ICV immunized cats became positive based on PCR analysis at 12 weeks post-challenge. This shows a potential utility for recombinants expressing Env.

In summary, prime/boost protocols involving priming with ALVAC recombinants followed by boosting with inactivated FIV-infected cell vaccines can elicit protective immunity against experimental challenge with heterologous FIV strains. This immunity is long lasting and also provides partial protection against distinctly heterologous FIV-strains of other subtypes. The data supports a role for cell mediated rather than viral neutralizing antibody responses and FIV-specific antibody responses. These findings are relevant not only to the development of multiple subtype FIV-vaccines but also to the development of effective multiple subtype HIV vaccines (as well as multiple subtype vaccines for other lentiviruses and other retroviruses) as new subtypes continue to arise and existing subtypes increasingly spread to new geographical areas.

A Fisher's exact test was performed. This is a modification of the Chi square test. This test should be used when comparing two sets of discontinuous, quantal (all or none) data. The analysis was set up as follows:

	Vaccinated	Unvaccinated
35 Infected	A	B
Uninfected	C	D

For a single tailed probability the P value is calculated as:

$$P(\text{probability}) = (A+B)! (C+D)! (A+C)! (B+D)! / N! A! B! C! D!$$

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Each group was compared to the ALVAC-control group (n=6) and to the ALVAC-control group +ALVAC-control&ICV group (n=9). A P value equal or less than 0.05 was considered significant.

Table 5: Viral neutralizing antibody titers upon immunization.

Vaccine	VN titer				
	Cat ID#	pre-	post-immunizations	post-challenge	
				3 mo.	12 mo.
Alvac-env	QU1	<5	<5	<5	
	PY1	<5	<5	>100	
Alvac-gag/prot	QX3	<5	<5	<5	<5
	QQ1	<5	<5	<5	<5
	QI1	<5	<5	<5	<5
	QL3	<5	<5	<5	<5
Alvac-env, gag/prot	QS4	<5	<5	>100	
	PY3	<5	<5	<5	
Alvac-env, gag/prot & ICV	QH3	<5	<5	5-20	<5
	QA3	<5	<5	5-20	<5
	PY4	<5	<5	5-20	5-20
ALVAC-control	QC4	<5	<5	>100	ND ^a
	PY4	<5	<5	>100	
	QA4	in prep			
	QE3	in prep			
ALVAC-control & ICV	QG4	<5	<5	>100	ND
	QC5	<5	<5	>100	
	QE4	<5	<5	>100	

^a ND - Not Determined

Table 6 Statistical significance of efficacy data.

vaccine	viral status			
	vaccine group +/-	control group +/- (control)	P value (Single- tailed)	significant
Alvac-env	3/3	4/2	0.5	no
		7/2	0.28	no
Alvac-gag/prot	0/6	4/2	0.0303	yes
		7/2	0.00914	yes
Alvac-env, gag/prot	2/4	4/2	0.28	no
		7/2	0.118	no
Alvac-97TMG	3/3	4/2	0.5	no
		7/2	0.28	no
Alvac-env, gag/prot IWC	0/3	3/0	0.05	yes
	0/3	7/2	0.00914	yes
All groups combined	8/19	7/2	0.0158	yes

Table 7 Parameters pre- and post-secondary challenge (FIV Bangston)

pre 2nd challenge WB titer	VN pre 2nd challenge ^a		VN post 2nd challenge ^b		post 2nd challenge ^b		Protection Rate
	FIVpet titer	FIVbang titer	FIVpet titer	FIVbang titer	WB titer	virus status RT/PCR	
QA6	<5	ND	>5	ND	+(3-4)	-/-	
QH3	<5	ND	>5	ND	+(3)	-/-	2/3
PY4	5-20	ND	>5	ND	+(3-4)	-/+	
GU5	<5	<5	ND	ND	+(4-5)	+/+	
DH3	<5	<5	ND	ND	+(5)	+/+	0/3
EJ2	<5	<5	ND	ND	+(4)	+/+	

^a Serum sample taken 8 months post challenge, at the day of 2nd challenge.^b Serum taken 4 months post 2nd-challenge.^c ND - Not Determined.

EXAMPLE 9 - Generation of Additional NYVAC & TROVAC Recombinants

Using the strategies outlined above for generating FIV coding DNA linked to a promoter, flanking
5 DNA for NYVAC and TROVAC for insertion into regions of these vectors, analogous to embodiments in U.S. Patent No. 5,494,807 and No. 5,863,542 are employed to generate NYVAC and TROVAC FIV recombinants. Analysis demonstrates incorporation into the vectors of the
10 exogenous DNA and of expression thereof. Such additional recombinants are useful in the same manner as the above-described ALVAC embodiments.

EXAMPLE 10 - Generation of Additional Lentivirus and Additional Vector System Recombinants

15 Using the strategies analogous to those outlined above for generating FIV coding DNA linked to a promoter and the strategies for generating alternative poxvirus, baculovirus, adenovirus, herpesvirus, alphavirus, poliovirus, Epstein-Barr, bacterial, and DNA-
20 based systems in the documents cited herein and the knowledge of coding DNA from lentiviruses, retroviruses or immunodeficiency viruses, e.g., EIAV, FIV, BIV, HIV, or SIV, from the documents cited herein, alternative poxvirus, baculovirus, adenovirus, herpesvirus,
25 alphavirus, poliovirus, Epstein-Barr, bacterial, and DNA-based recombinants containing and expressing DNA from lentiviruses, retroviruses or immunodeficiency viruses, e.g., EIAV, FIV, BIV, HIV, or SIV, such as Env, Gag and protease and Gag and protease, recombinants are
30 generated. Analysis demonstrates incorporation into the vectors of the exogenous DNA and of expression thereof. Such additional recombinants are useful in the same manner as the above-described ALVAC embodiments.

Having thus described in detail preferred
35 embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited by particular details set

forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope thereof.

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104.1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Virogenetics Corporation
- (ii) TITLE OF INVENTION: Poxvirus-Based Expression Vectors Containing
Heterologous Inserts Derived From
Lentiviruses
- (iii) NUMBER OF SEQUENCES: 37
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: MBM & CO.
 - (B) STREET: P.O. BOX 809, STATION B
 - (C) CITY: OTTAWA
 - (D) PROVINCE: ONTARIO
 - (E) COUNTRY: CANADA
 - (F) POSTAL CODE: K1P 5P9
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Ver. 2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 2,271,955
 - (B) FILING DATE: 1997-11-07
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/746,668
 - (B) FILING DATE: 1996-11-14
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: SWAIN, Margaret
 - (B) REGISTRATION NUMBER:
 - (C) REFERENCE/DOCKET NUMBER:
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 613/567-0762
 - (B) TELEFAX: 613/563-7671

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2571 base pairs
 - (B) TYPE: DNA
 - (C) STRANDEDNESS:

104.2

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Feline immunodeficiency virus

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

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ccatttaggg tacctggaat aacagaaaaa gaaaagcaaa actactgtaa catattacaa 180
cctaagttac aagatctaag gaacgaaatt caagaggtaa aactggaaga aggaaatgca 240
ggtaagttta gaagagcaag atttttaagg tattctgatg aacaagtatt gtccctgggtt 300
catgcgttca taggatattg tatatattta ggtaatcgaa ataagttagg atctttaaga 360
catgacattg atatagaagc accccaagaa gagtggttata ataatagaga gaaggggtaca 420
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ccaccattag tagtcccagt agaagaatca gaaataatth tttgggactg ttgggcacca 600
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104.3

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4616 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Feline Immunodeficiency virus

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

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104.5

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104.6

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104.7

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1615 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence:
ALVAC-nucleotide sequence comprised in
the C6 donor plasmid pC6L.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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104.8

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 gttaaaagct gcaataaata gtaaggatgt agaagaaata ctttggttcta taccttcgga 1560
 ggaaagaact ttagaacaac ttaagtttaa tcaaacttgt atttatgaag gtacc 1615

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2879 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence:
 predicted nucleotide sequence of vaccinia
 virus H6 promoter and FIV env in an
 expression cassette.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ttaaataaaa atacttactt acgaaaaatg actaattagc tataaaaacc cgggttcttt 60
 attctatact taaaaagtga aaataaatac aaagggttctt gaggggttggtg ttaaattgaa 120
 agcgagaaat aatcataaat tatttcatta tcgcatatc cggttaagttt gtatcgtaat 180
 ggcagaagga ttgacagcca atagacaatg gataggacca gaagaagctg aagagttatt 240

104.9

agattttgat atagcaacac aaatgagtga agaaggacca ctaaattccag gagtaaacc 300
 atttagggta cctggaataa cagaaaaaga aaagcaaac tactgtaaca tattacaacc 360
 taagttacaa gatctaagga acgaaattca agaggtaaaa ctggaagaag gaaatgcagg 420
 taagtttaga agagcaagat ttttaaggta ttctgatgaa caagtattgt ccctgggttca 480
 tgcgttcata ggatattgta tatatttagg taatcgaaat aagttaggat ctttaagaca 540
 tgacattgat atagaagcac cccaagaaga gtgttataat aatagagaga aggggtacaac 600
 tgacaatata aaatatggta gacgatgttg cctaggaacg gtgactttgt acctgatttt 660
 atttatagga ttaataatat attcacagac aaccaacgct caggtagtat ggagacttcc 720
 accattagta gtcccagtag aagaatcaga aataattttt tgggactgtt gggcaccaga 780
 agaaccgccc tgtcaggact ttcttggggc aatgatacat ctaaaagcta agacaaatat 840
 aagtatacga gagggaccta ccttggggaa ttggactaga gaaatatggg caacattatt 900
 caaaaaggct actagacaat gtagaagagg cagaatatgg aaaagatgga atgagactat 960
 aacaggacca tcaggatgtg ctaataacac atgttataat gtttcagtaa tagtacctga 1020
 ttatcagtgt tatttagata gagtagatac ttgggttaciaa gggaaaataa atatatcatt 1080
 atgtctaaca ggaggaaaaa tgttgtaciaa taaagttaca aaacaattaa gctattgtac 1140
 agaccatta caaatccac tgatcaatta tacatttgga cctaatacaa catgtatgtg 1200
 gaatacttca caaattcagg accctgaaat accaaaatgt ggatgggtgga atcaaatggc 1260
 ctattataac agttgtaaat gggaagaggc aaaggtaaag tttcattgtc aaagaacaca 1320
 gagtcagcct ggatcatggc gtagagcaat ctcgatcatg aaacaagaa atagatggga 1380
 gtggagacca gatttggaat gtaaaaagggt gaaaatatct ctacagtga atagcacaaa 1440
 aaacctaacc ttgcaatga gaagttcagg agattatgga gaagtaacgg gagcttgat 1500
 agagtttgga tgatcataga ataaatacaa acatcattct gaagcaagggt ttagaattag 1560
 atgtagatgg aatgtaggat ccgatacctc gctcattgat acatgtggaa acactcgaga 1620
 tgtttcagggt gcgaatcctg tagattgtac catgtattca aataaaatgt acaattgttc 1680
 ttacaaaat gggtttacta tgaaggtaga tgaccttatt gtgcatttca atatgacaaa 1740
 agctgtagaa atgtataata ttgctggaaa ttggtcttgt acatctgact tgccatcgtc 1800

104.10

atgggggtat atgaattgta attgtacaaa tagtagtagt agttatagtg gtactaaaat 1860
 ggcatgtcct agcaatcgag gcatcttaag gaattggtat aaccagtag caggattacg 1920
 acaatcctta gaacagtatc aagttgtaaa acaaccagat tacttagtgg tcccagagga 1980
 agtcatggaa tataaaccta gaaggaaaag ggcagctatt catgttatgt tggctcttgc 2040
 aacagtatta tctattgtcg gtgcagggac gggggctact gctataggga tggtaacca 2100
 ataccaccaa gttctggcaa cccatcaaga agctatagaa aaggtgactg aagccttaaa 2160
 gataaacaac ttaagattag ttacattaga gcatcaagta ctagtaatag gattaaagt 2220
 agaagctatg gaaaaattct tatatacagc tttcgctatg caagaattag gatgtaatca 2280
 aatcaattc ttctgcaaaa tccctcctgg gttgtggaca aggtataata tgactataaa 2340
 tcaaacaata tggaatcatg gaaatataac tttgggggaa tggataacc aaacaaaaga 2400
 ttacaacaa aagttttatg aaataataat ggacatagaa caaataatg tacaaggga 2460
 aacagggata caacaattac aaaagtggga agattgggta ggatggatgg gaaatattcc 2520
 acaatattta aagggactat tgggaggtat cttgggaata ggattaggag tgttattatt 2580
 gattttatgt ttacctacat tggttgattg tataagaaat tgtatccaca agatactagg 2640
 atacacagta attgcaatgc ctgaagtaga aggagaagaa atacaaccac aatggaatt 2700
 gaggagaaat ggtaggcaat gtggcatgtc tgaaaaagag gaggaatgat gaagtatctc 2760
 agaattcctg cagcccgggg gatccttaat taattagtta ttagacaagg tgaaaacgaa 2820
 actatttgta gcttaattaa ttagctgcag gaattctttt tattgattaa ctagtcaaa 2879

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2032 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:

104.11

(D) OTHER INFORMATION: Description of Artificial Sequence:
 predicted nucleotide sequence of vaccinia
 virus I3L promoter and FIV gag/protease in
 an expression cassette.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ttaatcaata aaaagaattc ctgcagccct gcagctaatt aattaagcta caaatagttt 60
cgttttcacc ttgtctaata actaattaat taaggatccc ccgtaccggg cccccctcg 120
aggtcgacat cgatacatca tgcagtgggt aaacaaaaac atttttattc tcaaattgaga 180
taaagtgaaa atatatatca ttatattaca aagtacaatt atttaggttt aatcatgggg 240
aatggacagg ggcgagattg gaaaatggcc attaagagat gtagtaatgt tgctgtagga 300
gtagggggga agagtaaaaa atttggagaa gggaatttca gatggggccat tagaatggct 360
aatgtatcta caggacgaga acctggtgat ataccagaga ctttagatca actaagggtg 420
gttatttgcg atttacaaga aagaagagaa aaatttggat ctagcaaaga aattgatatg 480
gcaattgtga cattaaaagt ctttgcggtg gcaggacttt tgaatatgac ggtgtctact 540
gctgctgcag ctgaaaatat gtatttctca atgggattag acactaggcc atctatgaaa 600
gaagcaggtg gaaaagagga aggccctcca caggcatatc ctattcaaac agtaaattgga 660
gtaccacaat atgtagcact tgacccaaaa atggtgtcca ttttcatgga aaaggcaaga 720
gaaggactag gaggggagga agttcaacta tggtttactg ctttctctgc aaatttaaca 780
cctactgaca tggccacatt aataatggcc gcaccagggt gcgctgcaga taaagaaata 840
ttggatgaaa gcttaaagca actgacagca gaatatgatc gcacacatcc ccctgatgct 900
cccagaccat taccctattt tactgcagca gaaattatgg gtataggatt aactcaagaa 960
caacaagcag aagcaagatt tgcaccagct aggatgcagt gtagagcatg gtatctcgag 1020
gcattaggaa aattggctgc cataaaagct aagtctcctc gagctgtgca gttaagacaa 1080
ggagctaagg aagattattc atcctttata gacagattgt ttgcccaaat agatcaagaa 1140
caaaatacag ctgaagttaa gttatattta aaacagtcac taagcatagc taatgctaata 1200
gcagactgta aaaaggcaat gagccacctt aagccagaaa gtaccctaga agaaaagttg 1260
agagcttgtc aagaaatagg ctcaccagga tataaaatgc aactcttggc agaagctctt 1320
acaaaagttc aagtagtgca atcaaaagga tcaggaccag tgtgttttta ttgtaaaaaa 1380
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104.12

ccaggacatc tagcaagaca atgtagagaa gtgaaaaaat gtaataaatg tggaaaacct 1440
 ggtcatctag ctgccaaatg ttggcaagga aatagaaaga attcgggaaa ctggaaggcg 1500
 gggcgagctg cagccccagt gaatcaaatg cagcaagcag taatgccatc tgcacctcca 1560
 atggaggaga aactattgga tttataaatt ataataaagt aggtacgact acaacattag 1620
 aaaagaggcc agaaatactt atatttgtaa atggatatcc tataaaattt ttattagata 1680
 caggagcaga tataacaatt ttaaatagga gagattttca agtaaaaaat tctatagaaa 1740
 atggaaggca aaatatgatt ggagtaggag gaggaagag aggaacaaat tatattaatg 1800
 tacatttaga gattagagat gaaaattata agacacaatg tatatttggt aatgtttgtg 1860
 tcttagaaga taactcatta atacaacat tattggggag agataatatg attaaattca 1920
 atattagggt agtaatggct caataatttt atcccgggt tttatagcta attagtcatt 1980
 tttcgtaagt aagtattttt atttaatact ttttattgta cttatgttaa at 2032

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4727 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence:
 predicted nucleotide sequence of vaccinia
 virus H6 promoter and FIV env, and
 vaccinia virus I3L promoter and FIV
 gag/protease in an expression cassette.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ttaatcaata aaaagaattc ctgcagccct gcagctaatt aattaagcta caaatagttt 60
 cgttttcacc ttgtctaata actaattaat taaggatccc ccgtaccggg cccccctcg 120
 aggtcgactt ctttattcta tacttaaaaa gtgaaaataa atacaaaggt tcttgagggt 180

104.13

tgtgttaaatt tgaaagcgag aaataatcat aaattatttc attatcgcgga tatccgttaa 240
 gtttgtatcg taatggcaga aggatttgca gccaatagac aatggatagg accagaagaa 300
 gctgaagagt tattagattt tgatatagca acacaaatga gtgaagaagg accactaaat 360
 ccaggagtaa acccatttag ggtacctgga ataacagaaa aagaaaagca aaactactgt 420
 aacatattac aacctaagtt acaagatcta aggaacgaaa ttcaagaggt aaaactggaa 480
 gaaggaaatg caggtaagtt tagaagagca agatttttaa ggtattctga tgaacaagta 540
 ttgtccctgg ttcatgcgtt cataggatat tgtatatatt taggtaatcg aaataagtta 600
 ggatctttta gacatgacat tgatatagaa gcacccaag aagagtgtta taataataga 660
 gagaagggtta caactgacaa tataaaatat ggtagacgat gttgcctagg aacgggtgact 720
 ttgtacctga ttttatttat aggattaata atatattcac agacaaccaa cgctcaggta 780
 gtatggagac ttccaccatt agtagtccca gtagaagaat cagaaataat tttttgggac 840
 tgttgggcac cagaagaacc cgctgtcag gactttcttg gggcaatgat acatctaaaa 900
 gctaagacaa atataagtat acgagaggga cctaccttgg ggaattggac tagagaaata 960
 tgggcaacat tattcaaaaa ggctactaga caatgtagaa gaggcagaat atggaaaaga 1020
 tggaatgaga ctataacagg accatcagga tgtgctaata acacatgtta taatgtttca 1080
 gtaatagtac ctgattatca gtgttattta gatagagtag atacttggtt acaagggaaa 1140
 ataaatatat cattatgtct aacaggagga aaaatgttgt acaataaagt taaaaacaa 1200
 ttaagctatt gtacagaccc attacaaatc ccactgatca attatacatt tggacctaat 1260
 caaacatgta tgtggaatac ttcacaaatt caggaccctg aaataccaaa atgtggatgg 1320
 tggaatcaaa tggcctatta taacagttgt aaatgggaag aggcaaagggt aaagtttcat 1380
 tgtcaaagaa cacagagtca gcctggatca tggcgtagag caatctcgtc atggaaacaa 1440
 agaaatagat gggagtggag accagatttg gaaagtaaaa aggtgaaaat atctctacag 1500
 tgcaatagca caaaaaacct aacctttgca atgagaagtt caggagatta tggagaagta 1560
 acgggagctt ggatagagtt tggatgtcat agaaataaat caaacatca ttctgaagca 1620
 aggttttagaa ttagatgtag atggaatgta ggatccgata cctcgctcat tgatacatgt 1680
 ggaaacactc gagatgtttc aggtgcgaat cctgtagatt gtaccatgta ttcaaataaa 1740

104.14

atgtacaatt gttctttaca aaatggggttt actatgaagg tagatgacct tattgtgcat 1800
 ttcaatatga caaaagctgt agaaatgtat aatattgctg gaaattggtc ttgtacatct 1860
 gacttgccat cgtcatgggg gtatatgaat tgtaattgta caaatagtag tagtagttat 1920
 agtgggtacta aaatggcatg tcctagcaat cgaggcatct taaggaattg gtataacca 1980
 gtagcaggat tacgacaatc cttagaacag tatcaagttg taaaacaacc agattactta 2040
 gtgggtcccag aggaagtcac ggaatataaa cctagaagga aaagggcagc tattcatgtt 2100
 atggttggtc ttgcaacagt attatctatt gtcggtgcag ggacgggggc tactgctata 2160
 gggatggtaa cccaatacca ccaagttctg gcaacccatc aagaagctat agaaaagggtg 2220
 actgaagcct taaagataaa caacttaaga ttagttacat tagagcatca agtactagta 2280
 ataggattaa aagtagaagc tatggaaaaa ttcttatata cagctttcgc tatgcaagaa 2340
 ttaggatgta atcaaaatca attcttctgc aaaatccctc ctgggttggtg gacaagggtat 2400
 aatatgacta taaatcaaac aatatggaat catggaaata taactttggg ggaatgggtat 2460
 aaccaaacaa aagatttaca acaaaagttt tatgaaataa taatggacat agaacaaaat 2520
 aatgtacaag ggaaaacagg gatacaacaa ttacaaaagt gggaagattg ggtaggatgg 2580
 atgggaaata ttccacaata tttaaaggga ctattgggag gtatcttggg aataggatta 2640
 ggagtgttat tattgatttt atgtttacct acattgggtg attgtataag aaattgtatc 2700
 cacaagatac taggatacac agtaattgca atgcctgaag tagaaggaga agaaatacaa 2760
 ccacaaatgg aattgaggag aaatggtagg caatgtggca tgtctgaaaa agaggaggaa 2820
 tgaatcgata catcatgcag tggttaaaca aaaacatttt tattctcaa tgagataaag 2880
 tgaaaatata tatcattata ttacaaagta caattattta gggtttaatca tggggaatgg 2940
 acaggggcca gattggaaaa tggccattaa gagatgtagt aatggtgctg taggagtagg 3000
 ggggaagagt aaaaaatttg gagaaggga tttcagatgg gccattagaa tggctaattg 3060
 atctacagga cgagaacctg gtgatatacc agagacttta gatcaactaa ggttggttat 3120
 ttgcgattta caagaaagaa gagaaaaatt tggatctagc aaagaaattg atatggcaat 3180
 tgtgacatta aaagtctttg cggtagcagg acttttgaat atgacggtgt ctactgctgc 3240
 tgcagctgaa aatatgtatt ctcaaaggg attagacact aggccatcta tgaaagaagc 3300

104.15

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aggtggaaaa gaggaaggcc ctccacaggc atatacctatt caaacagtaa atggagtacc 3360
acaatatgta gcacttgacc caaaaatgggt gtccatttttc atggaaaagg caagagaagg 3420
actaggaggg gaggaagttc aactatgggt tactgccttc tctgcaaatt taacacctac 3480
tgacatggcc acattaataa tggccgcacc aggggtgcgct gcagataaag aaatattgga 3540
tgaaagctta aagcaactga cagcagaata tgatcgaca catccccctg atgctcccag 3600
accattaccc tattttactg cagcagaaat tatgggtata ggattaactc aagaacaaca 3660
agcagaagca agatttgcac cagctaggat gcagtgtaga gcatggtatc tcgaggcatt 3720
aggaaaattg gctgccataa aagctaagtc tctcgcagct gtgcagttaa gacaaggagc 3780
taaggaagat tattcatcct ttatagacag attgtttgcc caaatagatc aagaacaaaa 3840
tacagctgaa gttaagttat atttaaaaca gtcattaagc atagctaata ctaatgcaga 3900
ctgtaaaaag gcaatgagcc accttaagcc agaaagtacc ctagaagaaa agttgagagc 3960
ttgtcaagaa ataggctcac caggatataa aatgcaactc ttggcagaag ctcttacaaa 4020
agttcaagta gtgcaatcaa aaggatcagg accagtgtgt ttttaattgta aaaaaccagg 4080
acatctagca agacaatgta gagaagtga aaaatgtaat aaatgtggaa aacctggtca 4140
tctagctgcc aaatgttggc aaggaaatag aaagaattcg ggaaactgga aggcggggcg 4200
agctgcagcc ccagtgaatc aaatgcagca agcagtaatg ccatctgcac ctccaatgga 4260
ggagaaacta ttggatttat aaattataat aaagtaggta cgactacaac attagaaaag 4320
aggccagaaa tacttatatt tgtaaattgga taccctataa aatttttatt agatacagga 4380
gcagatataa caatttttaa taggagagat tttcaagtaa aaaattctat agaaaatgga 4440
aggcaaaata tgattggagt aggaggagga aagagaggaa caaattatat taatgtacat 4500
ttagagatta gagatgaaaa ttataagaca caatgtatat ttggtaatgt ttgtgtctta 4560
gaagataact cattaatata accattattg gggagagata atatgattaa attcaatatt 4620
aggttagtaa tggctcaata attttatccc gggtttttat agctaattag tcatttttcg 4680
taagtaagta tttttattta atacttttta ttgtacttat gttaaatt 4727

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

104.16

- (A) LENGTH: 4150 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence:
predicted nucleotide sequence of vaccinia virus H6 promoter and altered FIV env, and vaccinia virus I3L promoter and FIV gag/pol in an expression cassette.

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

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ttaatcaata aaaagaattc ctgcaggaat tcataaaaat cattcttctc cttctacttc 60
aggcattgca attactgtgt atcctagtat cttgtggata caatttctta tacaatcaac 120
caatgtaggt aaacataaaa tcaataataa cactccta at cctattccca agatacctcc 180
caatagtc cc cttttccttc taggtttata ttccatgact tcctctggga ccactaagta 240
atctggttgt ttacaactt gatactgttc taaggattgt cgtaatcctg ctactggggtt 300
ataccaattc ctttaagatgc ctcgattgct aggacatgcc attttagtac cactataact 360
actactacta tttgtacaat tacaattcat atacccccat gacgatggca agtcagatgt 420
acaagaccaa tttccagcaa tattatacat ttctacagct tttgtcatat tgaaatgcac 480
aataagggtca tctaccttca tagtaaacc attttgtaaa gaacaattgt acattttatt 540
tgaatacatg gtacaatcta caggattcgc acctgaaaca tctcgagtgt ttccacatgt 600
atcaatgagc gaggtatcgg atcctacatt ccatctacat ctaattctaa accttgcttc 660
agaatgatgt tttgatttat ttctatgaca tccaaactct atccaagctc ccgttacttc 720
tccataatct cctgaacttc tcattgcaaa ggtaggttt tttgtgctat tgcactgtag 780
agatatatttc acctttttac ttccaaatc tgggtctccac tcccatctat ttctttgttt 840
ccatgacgag attgctctac gccatgatcc aggctgactc tgtgttcttt gacaatgaaa 900
ctttaccttt gcctcttccc atttacaact gttataatag gccatttgat tccaccatcc 960

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104.17

acatttttggg atttcagggg cctgaatttg tgaagtattc cacatacatg tttgattagg 1020
tccaaatgta taattgatca gtgggatttg taatgggtct gtacaatagc ttaattgttt 1080
tgtaacttta ttgtacaaca tttttcctcc tgtagacat aatgatatat ttattttccc 1140
ttgtaaccaa gtatctactc tatctaaata aactgataa tcagggtacta ttactgaaac 1200
attataacat gtgttattag cacatcctga tggtcctggt atagtctcat tccatctttt 1260
ccatattctg cctcttctac attgtctagt agcctttttg aataatggtg cccatatttc 1320
tctagtccaa tcccccaagg taggtccctc tcgtatactt atatttgtct tagcttttag 1380
atgtatcatt gcccgaagaa agtcctgaca ggcgggttct tctggtgcc aacagtccca 1440
aaaaattatt tctgattctt ctactgggac tactaatggg ggaagtctcc atactacctg 1500
agcgttggtt gtctgtgaat atattattaa tcctataaat aaaatcaggg acaaagtcac 1560
cgttcctagg caacatcgtc taccatattt tatattgtca gttgtaccct tctctctatt 1620
attataacac tcttcttggg gtgcttctat atcaatgtca tgtcttaaag atcctaactt 1680
atttcgatta cctaaatata tacaatatcc tatgaacgca tgaaccaggg acaatacttg 1740
ttcatcagaa taccttaaaa atcttgctct tctaaactta cctgcatttc cttcttccag 1800
ttttacctct tgaatttcgt tccttagatc ttgtaactta gggttgtaata tgttacagta 1860
gttttgcttt tctttttctg ttattccagg taccctaaat gggtttactc ctggatttag 1920
tggtccttct tcaactattt gtgttgctat atcaaaatct aataactctt cagcttcttc 1980
tggtcctatc cattgtctat tggctgcaaa tccttctgcc attacgatac aaacttaacg 2040
gatatcgcg taatgaaata atttatgatt atttctcgct ttcaatttaa cacaacctc 2100
aagaaccttt gtatttatth tcacttttta agtatagaat aaagaactgc agctaattaa 2160
ttaagctaca aatagtttcg ttttcacctt gtctaataac taattaatta aggatcccc 2220
gtaccggggc cccctcgag gtcgacatcg atacatcatg cagtgggtta acaaaaacat 2280
ttttattctc aatgagata aagtgaat atatatcatt atattacaaa gtacaattat 2340
ttaggtttta tcatggggaa tggacagggg cgagattgga aaatggccat taagagatgt 2400
agtaatgttg ctgtaggagt aggggggaag agtaaaaaat ttggagaagg gaatttcaga 2460
tgggccatta gaatggctaa tgtatctaca ggacgagaac ctggtgatat accagagact 2520

104.18

ttagatcaac taaggttggt tatttgcat ttacaagaaa gaagagaaaa atttggatct 2580
 agcaaagaaa ttgatatggc aattgtgaca ttaaaagtct ttgcggtagc aggacttttg 2640
 aatatgacgg tgtctactgc tgctgcagct gaaaatatgt attctcaaatt gggattagac 2700
 actaggccat ctatgaaaga agcagggtgga aaagaggaag gccctccaca ggcatatcct 2760
 attcaaacag taaatggagt accacaatat gtagcacttg acccaaaaat ggtgtccatt 2820
 ttcatggaaa aggcaagaga aggactagga ggggaggaag ttcaactatg gtttactgcc 2880
 ttctctgcaa atttaacacc tactgacatg gccacattaa taatggccgc accagggtgc 2940
 gctgcagata aagaaatatt ggatgaaagc ttaaagcaac tgacagcaga atatgatcgc 3000
 acacatcccc ctgatgctcc cagaccatta ccctatttta ctgcagcaga aattatgggt 3060
 ataggattaa ctcaagaaca acaagcagaa gcaagatttg caccagctag gatgcagtgt 3120
 agagcatggt atctcgaggc attaggaaaa ttggctgcca taaaagctaa gtctcctcga 3180
 gctgtgcagt taagacaagg agctaaggaa gattattcat cctttataga cagattgttt 3240
 gcccaaatag atcaagaaca aaatacagct gaagttaagt tatatttaaa acagtcatta 3300
 agcatagcta atgctaattgc agactgtaaa aaggcaatga gccaccttaa gccagaaagt 3360
 accctagaag aaaagttgag agcttgtcaa gaaataggct caccaggata taaaatgcaa 3420
 ctcttggcag aagctcttac aaaagttcaa gtagtgcaat caaaaggatc aggaccagtg 3480
 tgttttaatt gtaaaaaacc aggacatcta gcaagacaat gtagagaagt gaaaaaatgt 3540
 aataaatgtg gaaaacctgg tcatctagct gccaaatggt ggcaaggaaa tagaaagaat 3600
 tcgggaaact ggaaggcggg gcgagctgca gcccagtgat atcaaattgca gcaagcagta 3660
 atgccatctg cacctccaat ggaggagaaa ctattggatt tataaattat aataaagtag 3720
 gtacgactac aacattagaa aagaggccag aaatacttat atttgtaaatt ggatataccta 3780
 taaaattttt attagataga ggagcagata taacaatttt aaataggaga gattttcaag 3840
 taaaaaatc tatagaaaat ggaaggcaaa atatgattgg agtaggagga ggaaagagag 3900
 gaacaaatta tattaatgta catttagaga ttagagatga aaattataag acacaatgta 3960
 tatttggtaa tgtttgtgtc ttagaagata actcattaat acaaccatta ttggggagag 4020
 ataatatgat taaattcaat attagggttag taatggctca ataattttat cccgggtttt 4080

104.19

tatagctaat tagtcatttt tcgtaagtaa gtatttttat ttaatacttt ttattgtact 4140
 tatgttaa at 4150

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: sequence not described in specification

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with pMAW103 template, for construction of FIV expression plasmid pMAW103A.

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

atcatcaagc ttctgcagtt ctttattcta tactta 36

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 79 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the 3' part of FIV env in a reaction with ptg6184 template.

104.20

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

aaattcttat atacagcttt cgctatgcaa gaattaggat gtaatcaaaa tcaattcttc 60
 tgcaaaatcc ctcctgggt 79

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the 3' part of FIV env in a reaction with ptg6184 template.

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

cccatcgagt gcggctac

18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 75 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the 3' part of FIV env.

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

104.21

gcagaagaat tgattttgat tacatcctaa ttcttgcata gcgaaagctg tatataagaa 60
 tttttccata gcttc 75

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the 3' part of FIV env.

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

aagttctggc aacccatc

18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: dna
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used to amplify the vaccinia virus H6 promoter and the 5' part of FIV env in a reaction with pMM108 template.

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

atcatcctgc agaagcttcc cgggttcttt attctatact t

41

104.22

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used to amplify the vaccinia virus H6 promoter and the 5' part of FIV env in a reaction with pMM108 template.

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

ctgcaaattcc ttctgccatt acgatacaaa cttaac

36

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used to amplify the 3' part of the vaccinia virus H6 promoter and the 5' part of FIV env in a reaction with pMM122 template.

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

cgttaagttt gtatcgtaat ggcagaagga ttgcagcc

39

104.23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used to amplify the 3' part of the vaccinia virus H6 promoter and the 5' part of FIV env in a reaction with pMM122 template.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

cctcttgaat ttcgttcc

18

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with ALVAC template, for construction of insertion plasmid pC6L.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

atcatcgagc tcgcggccgc ctatcaaaag tcttaatgag tt

42

(2) INFORMATION FOR SEQ ID NO:18:

104.24

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with ALVAC template, for construction of insertion plasmid pC6L.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

gaattcctcg agctgcagcc cgggttttta tagctaatta gtcatttttt cgtaagtaag 60
 tattttttatt taa 73

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with ALVAC template, for construction of insertion plasmid pC6L.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

cccgggctgc agctcgagga attcttttta ttgattaact agtcaaataa gtatatataa 60
 ttgaaaaagt aa 72

104.25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with ALVAC template, for construction of insertion plasmid pC6L.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

gatgatggta ccttcataaa tacaagtttg attaaactta agttg

45

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 80 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: sequence added to insertion plasmid pC6L in construction of pMM117.

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

gggggatcct taattaatta gttattagac aaggtgaaaa cgaaactatt tgtagcttaa 60

ttaattagct gcaggaattc

80

104.26

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the central part of FIV gag in a reaction with ptg8133 template.

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

caaaaatggt gtccattttc atggaaaagg caagagaagg ac

42

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the central part of FIV gag in a reaction with ptg8133 template.

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

ctgctgcagt aaaatagg

18

104.27

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the 3' part of the vaccinia virus I3L promoter and the 5' part of FIV gag in a reaction with ptg8133 template.

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

cttctcttgc cttttccatg aaaatggaca ccatttttgg gtc

43

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the 3' part of the vaccinia virus I3L promoter and the 5' part of FIV gag in a reaction with ptg8133 template.

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

caattattta ggtttaataca tggggaatgg acaggggc

38

(2) INFORMATION FOR SEQ ID NO:26:

104.28

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the 3' part of the vaccinia virus I3L promoter and the 5' part of FIV gag in a reaction with pMM100 template.

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

cgcccctgtc cattccccat gattaaacct aaataattgt ac

42

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the 3' part of the vaccinia virus I3L promoter and the 5' part of FIV gag in a reaction with pMM100 template.

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

atcatcgctcg acatcgatac atcatgcagt ggttaaac

38

(2) INFORMATION FOR SEQ ID NO:28:

104.29

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE:DNA
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY:
- (vi) ORIGINAL SOURCE: Artificial Sequence
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify FIV protease in a reaction with pMM121 template.
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

caggacatct agcaagac

18

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE:DNA
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY:
- (vi) ORIGINAL SOURCE: Artificial Sequence
- (ix) FEATURE:
 - (A) NAME/KEY:
 - (B) LOCATION:
 - (C) IDENTIFICATION METHOD:
 - (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify FIV protease in a reaction with pMM121 template.
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

gatgatcccg ggataaaaat tattgagcca ttactaacct

40

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE:DNA
 - (C) STRANDEDNESS:

104.30

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the 3' part of FIV env in a reaction with pMM125#10 template.

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

tatgaattgt aattgtac

18

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 base pairs

(B) TYPE:DNA

(C) STRANDEDNESS:

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(C) IDENTIFICATION METHOD:

(D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the 3' part of FIV env in a reaction with pMM125#10 template.

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

gtagcataag gttaccgcgg ccgctaagct taggttacca tccctatagc agta

54

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE:DNA

(C) STRANDEDNESS:

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

104.31

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 5' PCR primer used to amplify the 3' part of FIV env in a reaction with pMM125#10 template.

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

gtagcataag gtaacctaa gcttagcggcc gcggtaaccc aataccacca agttctggc 59

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: 3' PCR primer used to amplify the 3' part of FIV env in a reaction with pMM125#10 template.

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

attaaccctc actaaag

17

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:

104.32

(D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with pMM125#10 template, for construction of FIV expression plasmid pMAW103.

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

acttgccatc gtcattggggg

20

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with pMM125#10 template, for construction of FIV expression plasmid pMAW103.

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

gatacctccc aatagtcctccc ttttccttct aggtttatat tc

42

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR primer used in a reaction with pMM125#10

104.33

template, for construction of FIV
expression plasmid pMAW103.

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

gaatataaac ctagaaggaa aaggggacta ttggggaggta tc

42

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE:DNA
- (C) STRANDEDNESS:
- (D) TOPOLOGY:

(vi) ORIGINAL SOURCE: Artificial Sequence

(ix) FEATURE:

- (A) NAME/KEY:
- (B) LOCATION:
- (C) IDENTIFICATION METHOD:
- (D) OTHER INFORMATION: Description of Artificial Sequence: PCR
primer used in a reaction with pMM125#10
template, for construction of FIV
expression plasmid pMAW103.

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

atcatcgaat tcataaaaat cattcttctc cttctacttc

40

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CLAIMS:

1. Use, for vaccinating a feline to induce a protective immune response against multiple subtypes of feline immunodeficiency virus (FIV), of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and a recombinant canarypox virus that comprises and expresses in vivo in a feline, a nucleic acid molecule that encodes one or more proteins selected from the group consisting of FIV Protease, FIV Env, the modified variant 97TM of FIV Env, and FIV Gag.
2. Use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response against multiple subtypes of feline immunodeficiency virus (FIV), of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and a recombinant canarypox virus that comprises and expresses in vivo in a feline, a nucleic acid molecule that encodes one or more proteins selected from the group consisting of FIV Protease, FIV Env, the modified variant 97TM of FIV Env, and FIV Gag.
3. The use according to claim 1 or 2 wherein the canarypox virus is an ALVAC virus.
4. Use, for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, a nucleic acid molecule encoding the FIV Env protein.
5. Use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response

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against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, a nucleic acid molecule encoding the FIV Env protein.

6. Use, for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env, Gag, and Protease proteins.

7. Use, in the preparation of a medicament for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env, Gag, and Protease proteins.

8. Use, for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Gag and FIV Protease proteins.

9. Use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Gag and FIV Protease proteins.

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10. Use, for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env modified variant 97TM, FIV Gag and FIV Protease proteins.

11. Use, in the manufacture of a medicament for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV, of an immunogenic composition comprising: a pharmaceutically or veterinarily acceptable carrier; and an ALVAC recombinant poxvirus that comprises and expresses in vivo in a feline, nucleic acid molecules encoding FIV Env modified variant 97TM, FIV Gag and FIV Protease proteins.

12. The use according to any one of claims 1, 4, 6, 8 and 10, further comprising use of an inactivated FIV cell vaccine.

13. A kit comprising: the immunogenic composition as defined in any one of claims 1, 2, or 3-11; and instructions for vaccinating a feline to induce a protective immune response against multiple subtypes of FIV.

14. The immunogenic composition as defined in any one of claims 1, 2, or 3-11, for the vaccination of a feline to induce a protective immune response against multiple subtypes of FIV.

15. A vector comprising exogenous DNA encoding at least one FIV epitope, wherein the vector is a canarypox virus.

16. The vector of claim 15, wherein the canarypox virus is the ALVAC strain, or is a Rentschler vaccine strain

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obtained by (a) attenuation of a Rentschler vaccine strain through more than 200 serial passages on chick embryo fibroblasts, (b) subjecting a master seed therefrom to four successive plaque purifications under agar, and (c) amplifying a plaque clone through five additional passages.

17. The vector of any one of claims 15 or 16, wherein the DNA that encodes at least one FIV epitope encodes all or part of (a) Gag-Pol, (b) Gag-protease, (c) Env, Gag-Pol, or (d) Env, Gag-protease.

18. The vector of any one of claims 15 or 16, wherein the DNA that encodes at least one FIV epitope encodes all or part of Gag-Pol or Gag-protease.

19. The vector of any one of claims 15 or 16, wherein the DNA that encodes at least one FIV epitope encodes all or part of Env, Gag-Pol, or Env, Gag-protease.

20. The vector of claim 15 which is vCP242, vCP253, vCP255, or vCP329, said vector comprising exogenous DNA encoding at least one FIV epitope, wherein vCP242, vCP253, vCP255, or vCP329 are recombinants of the ALVAC strain, wherein vCP242 comprises DNA encoding Env, vCP253 comprises DNA encoding Gag-protease, vCP255 comprises DNA encoding Env, Gag-protease, and wherein vCP329 comprises DNA encoding 97TM, Gag-protease.

21. A composition for inducing an immunological response comprising a vector as claimed in any one of claims 15-20 in admixture with a suitable carrier.

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PATENT AGENTS

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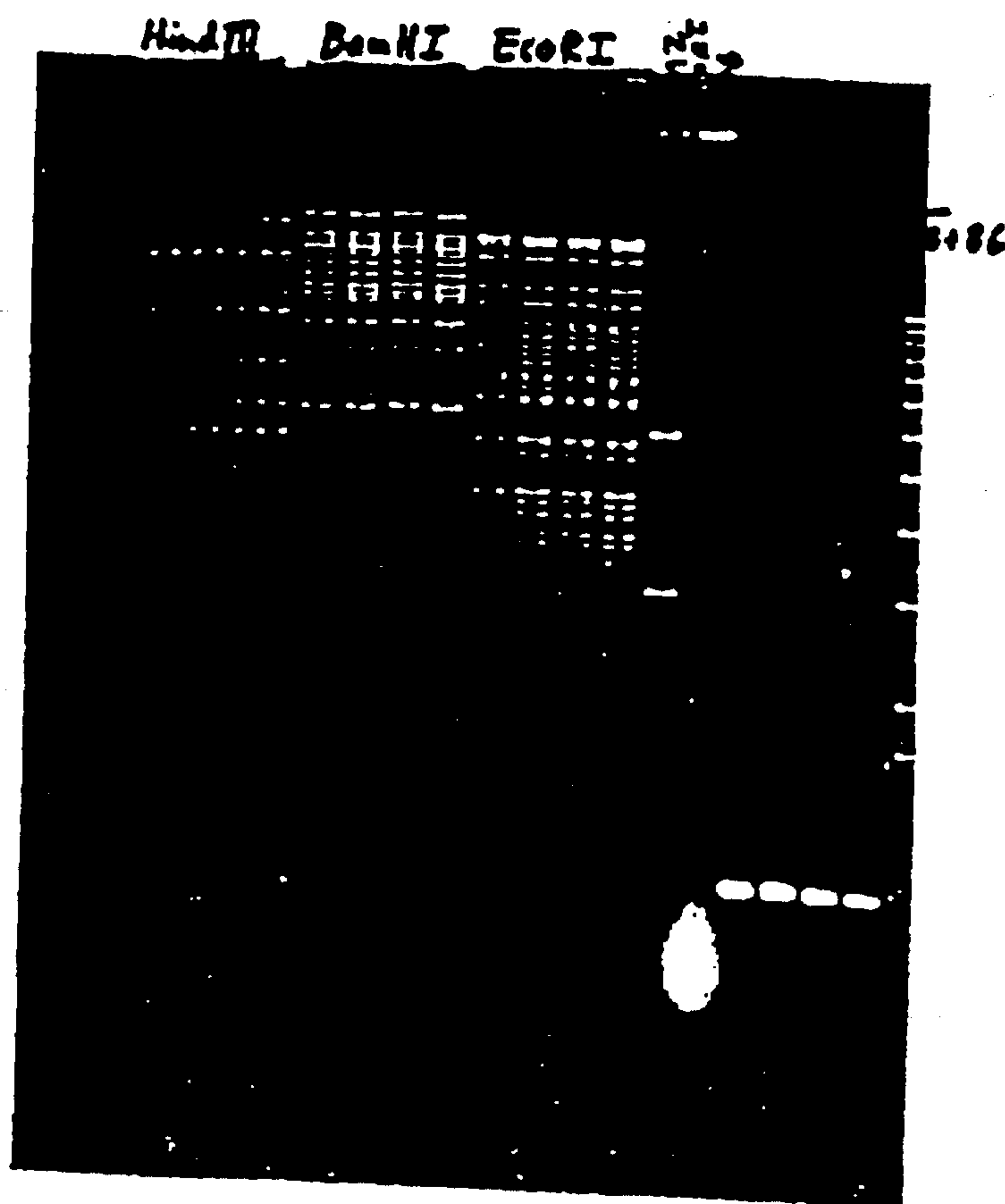


FIG. 1

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FIG. 2 Nucleotide sequence of FIV env from Rhone Merieux. The FIV env start codon is at position 1 and the stop codon is at position 2569. Plasmid ptg6184, containing the FIV env coding sequence, was from Rhone Merieux. The FIV env coding sequence in ptg6184 was sequenced and the following differences with the sequence below were observed: position 1218 T is G in ptg6184 changing phe to leu; position 1220 G to A changes gly to glu; and position 2201 C to A change ala to glu.

```
1   ATGGCAGAAGGATTTGCAGCCAATAGACAATGGATAGGACCAGAAGAAGCTGAAGAGTTA
61  TTAGATTTTGATATAGCAACACAAATGAGTGAAGAAGGACCACTAAATCCAGGAGTAAAC
121 CCATTTAGGGTACCTGGAATAACAGAAAAAGAAAAGCAAACTACTGTAACATATTACAA
181 CCTAAGTTACAAGATCTAAGGAACGAAATTCAAGAGGTAAAACCTGGAAGAAGGAAATGCA
241 GGTAAGTTTAGAAGAGCAAGATTTTTTAAGGTATTCTGATGAACAAGTATTGTCCCTGGTT
301 CATGCGTTCATAGGATATTGTATATATTTAGGTAATCGAAATAAGTTAGGATCTTTAAGA
361 CATGACATTGATATAGAAGCACCCCAAGAAGAGTGTTATAATAATAGAGAGAAGGGTACA
421 ACTGACAATATAAAATATGGTAGACGATGTTGCCTAGGAACGGTGACTTTGTACCTGATT
481 TTATTTATAGGATTAATAATATATTCACAGACAACCAACGCTCAGGTAGTATGGGAGACTT
541 CCACCATTAGTAGTCCCAGTAGAAGAATCAGAAATAATTTTTTGGGACTGTTGGGCACCA
601 GAAGAACCCGCCTGTCAGGACTTTCTTGGGGCAATGATACATCTAAAAGCTAAGACAAAT
661 ATAAGTATACGAGAGGGACCTACCTTGGGGAATTGGACTAGAGAAATATGGGCAACATTA
721 TTCAAAAAGGCTACTAGACAATGTAGAAGAGGCAGAATATGGAAAAGATGGAATGAGACT
781 ATAACAGGACCATCAGGATGTGCTAATAACACATGTTATAATGTTTCAGTAATAGTACCT
841 GATTATCAGTGTTATTTAGATAGAGTAGATACTTGGTTACAAGGGAAAATAAATATATCA
901 TTATGTCTAACAGGAGGAAAAATGTTGTACAATAAAGTTACAAAACAATTAAGCTATTGT
961 ACAGACCCATTACAAATCCCCTGATCAATTATACATTTGGACCTAATCAAACATGTATG
1021 TGAATACTTCACAAATTCAGGACCCTGAAATACCAAAATGTGGATGGTGGAAATCAAATG
1081 GCCTATTATAACAGTTGTAAATGGGAAGAGGCAGGTAAGTTTCATTGTCAAAGAACA
1141 CAGAGTCAGCCTGGATCATGGCGTAGAGCAATCTCGTCATGGAAACAAAGAAATAGATGG
1201 GAGTGGAGACCAGATTTTGGAAAGTAAAAAGGTGAAAATATCTCTACAGTGCAATAGCACA
1261 AAAAACCTAACCTTTGCAATGAGAAGTTCAGGAGATTATGGAGAAGTAACGGGAGCTTGG
1321 ATAGAGTTTGGATGTCATAGAAATAAATCAAAACATCATTCTGAAGCAAGGTTTAGAATT
1381 AGATGTAGATGGAATGTAGGATCCGATACCTCGCTCATTGATACATGTGGAAACACTCGA
1441 GATGTTTCAGGTGCGAATCCTGTAGATTGTACCATGTATTCAAATAAAATGTACAATTGT
1501 TCTTTACAAAATGGGTTTACTATGAAGGTAGATGACCTTATTGTGCATTTCAATATGACA
1561 AAAGCTGTAGAAATGTATAATATTGCTGGAAATTGGTCTTGACATCTGACTTGCCATCG
1621 TCATGGGGGTATATGAATTGTAATTGTACAAATAGTAGTAGTATAGTGGTACTAAA
1681 ATGGCATGTCCTAGCAATCGAGGCATCTTAAGGAATTGGTATAACCCAGTAGCAGGATTA
1741 CGACAATCCTTAGAACAGTATCAAGTTGTAAAACAACCAGATTACTTAGTGGTCCCAGAG
1801 GAAGTCATGGAATATAAACCTAGAAGGAAAAGGGCAGCTATTCATGTTATGTTGGCTCTT
1861 GCAACAGTATTATCTATTGTCGGTGCAGGGACGGGGGCTACTGCTATAGGGATGGTAACA
1921 CAATACCACCAAGTTCTGGCAACCCATCAAGAAGCTATAGAAAAGGTGACTGAAGCCTTA
1981 AAGATAAACAACTTAAGATTAGTTACATTAGAGCATCAAGTACTAGTAATAGGATTAAAA
2041 GTAGAAGCTATGGAAAAATTTTTATATACAGCTTTCGCTATGCAAGAATTAGGATGTAAT
2101 CAAAATCAATTTTTCTGCAAAATCCCTCCTGGGTTGTGGACAAGGTATAATATGACTATA
2161 AATCAAACAATATGGAATCATGGAAATATAACTTTGGGGGCATGGTATAACCAAACAAAA
2221 GATTTACAACAAAAGTTTATGAAATAATAATGGACATAGAACAAAATAATGTACAAGGG
2281 AAAACAGGGATACAACAATTACAAAAGTGGGAAGATTGGGTAGGATGGATGGGAAATATT
2341 CCACAATATTTAAAGGGACTATTGGGAGGTATCTTGGGAATAGGATTAGGAGTGTTATTA
2401 TTGATTTTATGTTTACCTACATTGGTTGATTGTATAAGAAATTGTATCCACAAGATACTA
2461 GGATACACAGTAATTGCAATGCCTGAAGTAGAAGGAGAAGAAATACAACCACAAATGGAA
2521 TTGAGGAGAAATGGTAGGCAATGTGGCATGTCTGAAAAAGAGGAGGAATGA
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FIG. 3 Nucleotide sequence of FIV gag/pol coding sequences from Rhone Merieux. The gag start codon is at position 1 and the gag stop codon is at position 1414. The ribosomal frameshift site is near position 1255. The frameshift is -1 in relation to the gag open reading frame. The frameshift goes into the pol open reading frame. The pol stop codon is at position 4614. Plasmid ptg8133 from Rhone Merieux contains the FIV gag/pol coding sequences. Part of ptg8133 has been sequenced and the CG at positions 577-578 below is GC in ptg8133, changing the codon from arg to ala.

```

1   ATGGGGAATGGACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGTAGTAATGTTGCT
61  GTAGGAGTAGGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTTCAGATGGGCCATTAGA
121 ATGGCTAATGTATCTACAGGACGAGAACCTGGTGATATAACCAGAGACTTTAGATCAACTA
181 AGGTTGGTTATTTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCTAGCAAAGAAATT
241 GATATGGCAATTGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTGAATATGACGGTG
301 TCTACTGCTGCTGCAGCTGAAAATATGTATTCTCAAATGGGATTAGACACTAGGCCATCT
361 ATGAAAGAAGCAGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCTATTCAAACAGTA
421 AATGGAGTACCACAATATGTAGCACTTGACCCAAAAATGGTGTCCATTTTTATGGAAAAG
481 GCAAGAGAAGGACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCCTTCTCTGCAAAT
541 TTAACACCTACTGACATGGCCACATTAATAATGGCCCGACCAGGGTGGCTGCAGATAAA
601 GAAATATTGGATGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGCACACATCCCCCT
661 GATGCTCCCAGACCATTACCCTATTTTACTGCAGCAGAAATTATGGGTATAGGATTAAGT
721 CAAGAACAACAAGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGAGCATGGTAT
781 CTCGAGGCATTAGGAAAATTTGGCTGCCATAAAAGCTAAGTCTCCTCGAGCTGTGCAGTTA
841 AGACAAGGAGCTAAGGAAGATTATTCATCCTTTATAGACAGATTGTTTGCCCAAATAGAT
901 CAAGAACAAAATACAGCTGAAGTTAAGTTATATTTAAACAGTCATTAAGCATAGCTAAT
961 GCTAATGCAGACTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCTAGAAGAA
1021 AAGTTGAGAGCTTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAACTCTTGGCAGAA
1081 GCTCTTACAAAAGTTCAAGTAGTGCAATCAAAAGGATCAGGACCAGTGTGTTTAAATTGT
1141 AAAAAACCAGGACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGTAATAAATGTGGA
1201 AAACCTGGTCATCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAATTGCGGAAACTGG
1261 AAGGCGGGGCGAGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTAATGCCATCTGCA
1321 CCTCCAATGGAGGAGAACTATTGGATTTATAAATTATAATAAAGTAGGTACGACTACAA
1381 CATTAGAAAAGAGGCCAGAAATACTTATATTTGTAAATGGATATCCTATAAAATTTTTAT
1441 TAGATACAGGAGCAGATATAACAATTTTAAATAGGAGAGATTTTCAAGTAAAAAATTCTA
1501 TAGAAAATGGAAGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAGGAACAAATTATA
1561 TTAATGTACATTTAGAGATTAGAGATGAAAATTATAAGACACAATGTATATTTGGTAATG
1621 TTTGTGTCTTAGAAGATAACTCATTAATACAACCATTATTGGGGAGAGATAATATGATTA
1681 AATTCAATATTAGGTTAGTAATGGCTCAAATTTCTGATAAGATTCCAGTAGTAAAGTAA
1741 AAATGAAGGATCCTAATAAAGGACCTCAAATAAAACAATGGCCATTAACAAATGAAAAAA
1801 TTGAAGCCTTAACAGAAATAGTAGAAAGACTAGAAAGAGAAGGGAAAGTAAAAAGAGCAG
1861 ATCCAAATAATCCATGGAATACACCAGTATTTGCTATAAAAAAGAAAAGTGGAAAATGGA
1921 GAATGCTCATAGATTTTAGAGAATTAAACAACTAACTGAGAAAGGAGCAGAGGTCCAGT
1981 TGGGACTACCTCATCCTGCTGGGTTACAAATAAAAAACAAGTAACAGTATTAGATATAG
2041 GGGATGCATATTTCAACCATTCCTCTTGATCCAGATTATGCTCCTTATACAGCATTTACTT
2101 TACCTAGGAAAAATAATGCGGGACCAGGAAGGAGATTTGTGTGGTGTAGTCTACCACAAG
2161 GCTGGATTTTAAGTCCATTGATATATCAAAGTACATTAGATAATATAATACAACCTTTTA
2221 TTAGACAAAATCCTCAATTAGATATTTACCAATATATGGATGACATTTATATAGGATCAA
2281 ATTTAAGTAAAAAGGAGCATAAAGAAAAGGTAGAAGAATTAAGAAAATTACTATTATGGT
2341 GGGGATTTGAACTCCAGAAGATAAATTACAGGAAGAACCCCCATATACATGGATGGGTT
2401 ATGAATTACATCCATTAACATGGACAATACAACAGAAACAGTTAGACATTCCAGAACAGC
2461 CCACTCTAATGAGTTGCAAAAATTAGCAGGAAAAATTAATTGGGCTAGCCAAGCTATTC

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FIG. 3 (cont'd)

2521 CAGACTTGAGTATAAAAGCATTAACTAACATGATGAGAGGAAATCAAAACCTAAATTCAA
2581 CAAGACAATGGACTAAAGAAGCTCGACTGGAAGTACAAAAGGCAAAAAGGCTATAGAAG
2641 AACAGTACAACCTAGGATACTATGACCCCAGTAAGGAGTTATATGCTAAATTAAGTTTGG
2701 TGGGACCACATCAAATAAGTTATCGAGTATATCAGAAGGATCAAGAAAAGATACTATGGT
2761 ATGGAAAAATGAGTAGACAAAAGAAAAAGGCAGAAAATACATGTGATATAGCCTTAAGAG
2821 CATGCTATAAGATAAGAGAAGAGTCTATTATAAGAATAGGAAAAGAACCAAGATATGAAA
2881 TACCTACTTCTAGAGAAGCCTGGGAATCAAATCTAATTAATTCACCATATCTTAAGGCC
2941 CACCTCCTGAGGTAGAATATATCCATGCTGCTTTGAATATAAAGAGAGCGTTAAGTATGA
3001 TAAAAGATGCTCCAATACCAGGAGCAGAAACATGGTATATAGATGGAGGTAGAAAACCTAG
3061 GAAAAGCAGCAAAAGCAGCCTATTGGACAGATACAGGAAAGTGGAAGTGATGGAATTAG
3121 AAGGCAGTAATCAGAAGGCAGAAATACAAGCATTATTATTGGCATTAAAAGCAGGATCAG
3181 AGGAGATGAATATTATAACAGATTCACAATATGCTATAAATATTATTCTTCAACAACCAG
3241 ATATGATGGAGGGAATCTGGCAAGAAGTTTTAGAGAATTGGAGAAGAAAACAGCAATAT
3301 TTATAGATTGGGTCCCAGGACATAAAGGTATTCCAGGAAATGAGGAAGTAGATAAGCTTT
3361 GTCAAACAATGATGATAATAGAAGGGGATGGGATATTAGACAAAAGGTCAGAAGATGCAG
3421 GATATGATTTATTAGCTGCAAAAGAAATACATTTATTGCCAGGAGAGGTAAAAGTAATAC
3481 CAACAGGGGTAAAGCTAATGCTGCCTAAAGGACATTGGGGATTAATAATCGGAAAAAGCT
3541 CGATGGGGAGTAAAGGATTGGATGTATTAGGAGGAGTAATAGATGAAGGATATCGAGGTG
3601 AAATTGGAGTAATAATGATTAATGTATCAAGAAAATCAATCACCTTAATGGAACGACAAA
3661 AGATAGCACAAATTAATAACTGCCTTGTAACATGAAGTATTAGAACAAGGAAAAGTAG
3721 TAAGGGATTCAGAGAGAGGAGGCAATGGTTATGGGTCAACAGGAGTATTCTCCTCTTGGG
3781 TTGACAGAATTGAGGAAGCAGAAATAAATCATGAAAAATTTCACTCAGATCCACAGTACT
3841 TAAGGACTGAATTTAATTTACCTAAAATGGTAGCAGAAGAGATAAGACGAAAATGCCAG
3901 TATGCAGAATCAGAGGAGAACAAGTGGGAGGACAATTGAAAATAGGGCCTGGTATCTGGC
3961 AAATGGATTGCACACACTTTGATGGCAAAATAATTCTTGTGGGTATACATGTGGAATCAG
4021 GATATATATGGGCACAAATAATTTCTCAAGAACTGCTGACTGTACAGTTAAAGCTGTTT
4081 TACAATTGTTGAGTGCTCATAATGTTACTGAATTACAAACAGATAATGGACCAAATTTTA
4141 AAAATCAAAAGATGGAAGGAGTACTCAATTACATGGGTGTGAAACATAAGTTTGGTATCC
4201 CAGGGAACCCACAGTCACAAGCATTAGTTGAAAATGTAAATCATAATTAAAAGTTTGGG
4261 TTCGGAAATTTTTGCCTGAAACAACCTCCTTGGATAATGCCTTATCTCTCGCTGTACATA
4321 GTCTCAATTTTAAAAGAAGAGGTAGGATAGGAGGGATGGCCCCTTATGAATTATTAGCAC
4381 AACAGAATCCTTAAGAATACAAGATTATTTTTCTGCAATACCACAAAATTGCAAGCAC
4441 AGTGGATTTATTATAAAGATCAAAAAGATAAGAAATGGAAAGGACCAATGAGAGTAGAAT
4501 ACTGGGGACAGGGATCAGTATTATTAAAGGATGAAGAGAAGGGATATTTTCTTATACCTA
4561 GGAGACACATAAGGAGAGTTCCAGAACCCTGCGCTCTTCTGAAGGGGATGAGTGA

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FIG. 4 Sequence comprised in the C6 donor plasmid pC6L. Plasmid pC6L contains the C6 insertion sites SmaI (position 409) and EcoRI (position 425).

```
1   GAGCTCGCGGCCGCCTATCAAAAGTCTTAATGAGTTAGGTGTAGATAGTATAGATATTAC
61  TACAAAGGTATTCATATTTCTTATCAATTCTAAAGTAGATGATATTAATAACTCAAAGAT
121 GATGATAGTAGATAATAGATACGCTCATATAATGACTGCAAATTTGGACGGTTCACATTT
181 TAATCATCACGCGTTCATAAGTTTCAACTGCATAGATCAAAATCTCACTAAAAAGATAGC
241 CGATGTATTTGAGAGAGATTGGACATCTAACTACGCTAAAGAAATTACAGTTATAAATAA
301 TACATAATGGATTTTGTATCATCAGTTATATTTAACATAAGTACAATAAAAAGTATTAA
361 ATAAAAATACTTACTTACGAAAAAATGACTAATTAGCTATAAAAACCCGGGCTGCAGCTC
421 GAGGAATTCTTTTTTATTGATTAAGTCAATGAGTATATATAATTGAAAAAGTAAAT
481 ATAAATCATATAATAATGAAACGAAATATCAGTAATAGACAGGAAGTGGCAGATTCTTCT
541 TCTAATGAAGTAAGTACTGCTAAATCTCCAAAATTAGATAAAAATGATACAGCAAATACA
601 GCTTCATTCAACGAATTACCTTTTAATTTTTTCAGACACACCTTATTACAACTAACTAA
661 GTCAGATGATGAGAAAGTAAATATAAATTTAACTTATGGGTATAATATAATAAGATTCA
721 TGATATTAATAATTTACTTAACGATGTTAATAGACTTATTCCATCAACCCCTTCAAACCT
781 TTCTGGATATTATAAAATACCAGTTAATGATATTAAAATAGATTGTTTAAGAGATGTAAA
841 TAATTATTTGGAGGTAAAGGATATAAAATTAGTCTATCTTTCACATGGAAATGAATTACC
901 TAATATTAATAATTATGATAGGAATTTTTTAGGATTTACAGCTGTTATATGTATCAACAA
961 TACAGGCAGATCTATGGTTATGGTAAACACTGTAACGGGAAGCAGCATTCTATGGTAAC
1021 TGGCCTATGTTTAATAGCCAGATCATTTTACTCTATAAACATTTTACCACAAATAATAGG
1081 ATCCTCTAGATATTTAATATTATATCTAACAACAACAAAAAATTTAACGATGTATGGCC
1141 AGAAGTATTTTCTACTAATAAAGATAAAGATAGTCTATCTTATCTACAAGATATGAAAGA
1201 AGATAATCATTTAGTAGTAGCTACTAATATGGAAAGAAATGTATACAAAAACGTGGAAGC
1261 TTTTATATTAAATAGCATATTACTAGAAGATTTAAATCTAGACTTAGTATAACAAAACA
1321 GTTAAATGCCAATATCGATTCTATATTTTCATCATAACAGTAGTACATTAATCAGTGATAT
1381 ACTGAAACGATCTACAGACTCAACTATGCAAGGAATAAGCAATATGCCAATTATGTCTAA
1441 TATTTTAACTTTAGAACTAAAACGTTCTACCAATACTAAAAATAGGATACGTGATAGGCT
1501 GTTAAAAGCTGCAATAAATAGTAAGGATGTAGAAGAAATACTTTGTTCTATACCTTCGGA
1561 GGAAAGAACTTTAGAACAACTTAAGTTTAATCAAACCTTGATTTTATGAAGGTACC
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FIG. 5 Predicted nucleotide sequence of the vCP242 insertion. The H6 promoter starts at position 55. The FIV env start codon is at position 179, and the FIV env stop codon is at position 2749. Positions 1 through 54 and positions 2750 through 2879 flank the H6/FIV env expression cassette.

```

1   TTAAATAAAAATACTTACTTACGAAAAATGACTAATTAGCTATAAAAACCCGGGTTCTTT
61  ATTCTATACTTAAAAAGTGAAAAATAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAA
121 AGCGAGAAATAATCATAAATTATTTTCATTATCGCGATATCCGTTAAGTTTGTATCGTAAT
181 GGCAGAAGGATTTGCAGCCAATAGACAATGGATAGGACCAGAAGAAGCTGAAGAGTTATT
241 AGATTTTGATATAGCAACACAAATGAGTGAAGAAGGACCACTAAATCCAGGAGTAAACCC
301 ATTTAGGGTACCTGGAATAACAGAAAAAGAAAAGCAAACTACTGTAACATATTACAACC
361 TAAGTTACAAGATCTAAGGAACGAAATTCAAGAGGTAAAACCTGGAAGAAGGAAATGCAGG
421 TAAGTTTAGAAGAGCAAGATTTTAAAGGTATTCTGATGAACAAGTATTGTCCCTGGTTCA
481 TCGGTTTCATAGGATATTGTATATATTTAGGTAATCGAAATAAGTTAGGATCTTTAAGACA
541 TGACATTGATATAGAAGCACCCCAAGAAGAGTGTTATAATAATAGAGAGAAGGGTACAAC
601 TGACAATATAAAATATGGTAGACGATGTTGCCTAGGAACGGTGACTTTGTACCTGATTTT
661 ATTTATAGGATTAATAATATATTCACAGACAACCAACGCTCAGGTAGTATGGGAGACTTCC
721 ACCATTAGTAGTCCCAGTAGAAGAATCAGAAATAATTTTTTGGGACTGTTGGGCACCAGA
781 AGAACCCGCTGTGTCAGGACTTTCTTGGGGCAATGATACATCTAAAAGCTAAGACAAATAT
841 AAGTATACGAGAGGGACCTACCTTGGGGAATTGGACTAGAGAAATATGGGCAACATTATT
901 CAAAAGGCTACTAGACAATGTAGAAGAGGCAGAATATGGAAAAGATGGAATGAGACTAT
961 AACAGGACCATCAGGATGTGCTAATAACACATGTTATAATGTTTCAGTAATAGTACCTGA
1021 TTATCAGTGTTATTTAGATAGAGTAGATACTTGGTTACAAGGGAAAATAAATATATCATT
1081 ATGTCTAACAGGAGGAAAAATGTTGTACAATAAAGTTACAAAACAATTAAGCTATTGTAC
1141 AGACCCATTACAAATCCCCTGATCAATTATACATTTGGACCTAATCAAACATGTATGTG
1201 GAATACTTCACAAATTCAGGACCCTGAAATACCAAAATGTGGATGGTGGAAATCAAATGGC
1261 CTATTATAACAGTTGTAAATGGGAAGAGGCCAAAGGTAAAGTTTCATTGTCAAAGAACACA
1321 GAGTCAGCCTGGATCATGGCGTAGAGCAATCTCGTCATGGAAACAAAGAAATAGATGGGA
1381 GTGGAGACCAGATTTGGAAAGTAAAAAGGTGAAAATATCTCTACAGTGCAATAGCACAAA
1441 AAACCTAACCTTTGCAATGAGAAGTTCAGGAGATTATGGAGAAGTAACGGGAGCTTGGAT
1501 AGAGTTTGGATGTCATAGAAATAAATCAAAACATCATTCTGAAGCAAGGTTTAGAATTAG
1561 ATGTAGATGGAATGTAGGATCCGATACCTCGCTCATTGATACATGTGGAAACACTCGAGA
1621 TGTTTCAGGTGCGAATCCTGTAGATTGTACCATGTATTCAAATAAAATGTACAATTGTTC
1681 TTTACAAAATGGGTTTACTATGAAGGTAGATGACCTTATTGTGCATTTCAATATGACAAA
1741 AGCTGTAGAAATGTATAATATTGCTGGAAATTGGTCTTGTACATCTGACTTGCCATCGTC
1801 ATGGGGGTATATGAATTGTAATTGTACAAATAGTAGTAGTATAGTGGTACTAAAAT
1861 GGCATGTCCTAGCAATCGAGGCATCTTAAGGAATTGGTATAACCCAGTAGCAGGATTACG
1921 ACAATCCTTAGAACAGTATCAAGTTGTAAAACAACCAGATTACTTAGTGGTCCCAGAGGA
1981 AGTCATGGAATATAAACCTAGAAGGAAAAGGGCAGCTATTCATGTTATGTTGGCTCTTGC
2041 AACAGTATTATCTATTGTGCGGTGCAGGGACGGGGGCTACTGCTATAGGGATGGTAACCCA
2101 ATACCACCAAGTTCTGGCAACCCATCAAGAAGCTATAGAAAAGGTGACTGAAGCCTTAAA
2161 GATAACAACCTTAAGATTAGTTACATTAGAGCATCAAGTACTAGTAATAGGATTAAAAGT
2221 AGAAGCTATGGAAAAATTCTTATATACAGCTTTCGCTATGCAAGAATTAGGATGTAATCA
2281 AAATCAATTCTTCTGCAAAATCCCTCCTGGGTTGTGGACAAGGTATAATATGACTATAAA
2341 TCAAACAATATGGAATCATGGAAATATACTTTGGGGGAATGGTATAACCAAACAAAAGA
2401 TTTACAACAAAAGTTTTATGAAATAATAATGGACATAGAACAAAATAATGTACAAGGGAA
2461 AACAGGGATACAACAATTACAAAAGTGGGAAGATTGGGTAGGATGGATGGGAAATATTCC
2521 ACAATATTTAAAGGGACTATTGGGAGGTATCTTGGGAATAGGATTAGGAGTGTTATTATT
2581 GATTTTATGTTTACCTACATTGGTTGATTGTATAAGAAATTGTATCCACAAGATACTAGG
2641 ATACACAGTAATTGCAATGCCTGAAGTAGAAGGAGAAGAAATACAACCACAAATGGAATT
2701 GAGGAGAAATGGTAGGCAATGTGGCATGTCTGAAAAAGAGGAGGAATGATGAAGTATCTC
2761 AGAATTCCTGCAGCCCGGGGGATCCTTAATTAATTAGTTATTAGACAAGGTGAAAACGAA
2821 ACTATTTGTAGCTTAATTAATTAGCTGCAGGAATTCTTTTTATTGATTAAGTACAAA

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FIG. 6 Predicted nucleotide sequence of I3L promoted FIV gag/protease expression cassette and flanking regions in vCP253. The I3L promoter begins at position 135. The gag start codon is at position 235 and the protease stop codon is at position 1648.

```
1   TTAATCAATAAAAAGAATTCCTGCAGCCCTGCAGCTAATTAATTAAGCTACAAATAGTTT
61  CGTTTTTCACCTTGTCTAATAACTAATTAATTAAGGATCCCCCGTACCGGGCCCCCCTCG
121 AGGTCGACATCGATACATCATGCAGTGGTTAAACAAAAACATTTTTATTCTCAAATGAGA
181 TAAAGTGAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAATCATGGGG
241 AATGGACAGGGGCGAGATTGGAATAATGGCCATTAAGAGATGTAGTAATGTTGCTGTAGGA
301 GTAGGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTTCAGATGGGCCATTAGAATGGCT
361 AATGTATCTACAGGACGAGAACCTGGTGATATACCAGAGACTTTAGATCAACTAAGGTTG
421 GTTATTTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCTAGCAAAGAAATTGATATG
481 GCAATTGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTGAATATGACGGTGTCTACT
541 GCTGCTGCAGCTGAAAATATGTATTCTCAAATGGGATTAGACACTAGGCCATCTATGAAA
601 GAAGCAGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCTATTCAAACAGTAAATGGA
661 GTACCACAATATGTAGCACTTGACCCAAAAATGGTGTCCATTTTCATGGAAAAGGCAAGA
721 GAAGGACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCCTTCTCTGCAAATTTAACA
781 CCTACTGACATGGCCACATTAATAATGGCCGCACCAGGGTGCGCTGCAGATAAAGAAATA
841 TTGGATGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGCACACATCCCCCTGATGCT
901 CCCAGACCATTACCCTATTTTACTGCAGCAGAAATTATGGGTATAGGATTAAGTCAAGAA
961 CAACAAGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGAGCATGGTATCTCGAG
1021 GCATTAGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGAGCTGTGCAGTTAAGACAA
1081 GGAGCTAAGGAAGATTATTCATCCTTTATAGACAGATTGTTTGCCCAAATAGATCAAGAA
1141 CAAAATACAGCTGAAGTTAAGTTATTTTAAAACAGTCATTAAGCATAGCTAATGCTAAT
1201 GCAGACTGTAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCTAGAAGAAAAGTTG
1261 AGAGCTTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAACTCTTGGCAGAAGCTCTT
1321 ACAAAGTTCAAGTAGTGCAATCAAAGGATCAGGACCAGTGTGTTTTAATTGTAAAAAA
1381 CCAGGACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGTAATAAATGTGGAAAACCT
1441 GGTCATCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAATTCGGGAAACTGGAAGGCG
1501 GGGCGAGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTAATGCCATCTGCACCTCCA
1561 ATGGAGGAGAACTATTGGATTTATAAATTATAATAAAGTAGGTACGACTACAACATTAG
1621 AAAAGAGGCCAGAAATACTTATATTTGTAAATGGATATCCTATAAAATTTTTATTAGATA
1681 CAGGAGCAGATATAACAATTTTAAATAGGAGAGATTTTCAAGTAAAAAATTTCTATAGAAA
1741 ATGGAAGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAGGAACAAATTATATTAATG
1801 TACATTTAGAGATTAGAGATGAAAATTATAAGACACAATGTATATTTGGTAATGTTTGTG
1861 TCTTAGAAGATAACTCATTAATACAACCATTATTGGGGAGAGATAATATGATTAAATTCA
1921 ATATTAGGTTAGTAATGGCTCAATAATTTTATCCCGGGTTTTTATAGCTAATTAGTCATT
1981 TTTCGTAAGTAAGTATTTTTATTTAATACTTTTTATTGTACTTATGTTAAT
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FIG. 7 Predicted nucleotide sequence of the H6 promoted FIV env/I3L promoted FIV gag/protease expression cassette and flanking regions in vCP255. The H6 promoter starts at position 129, the FIV env start codon is at position 253, and the FIV env stop codon is at position 2823. The I3L promoter starts at position 2830, the FIV gag start codon is at position 2930 and the FIV gag stop codon is at position 4282. The ribosomal frameshift site is near position 4184. The frameshift is -1 in relation to the gag open reading frame. The frameshift goes into the pol open reading frame. The stop codon for the protease gene is at position 4641. Positions 1 through 128 and positions 4642 through 4727 flank the H6 FIV env/I3L FIV gag/protease expression cassette.

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1   TTAATCAATAAAAAGAATTCCTGCAGCCCTGCAGCTAATTAATTAAGCTACAAATAGTTT
61  CGTTTTTCACCTTGTCTAATACTAATTAATTAAGGATCCCCCGTACCGGGCCCCCCCCTCG
121 AGGTCGACTTCTTTATTCTATACTTAAAAAGTGAAAATAAATACAAAGGTTCTTGAGGGT
181 TGTGTTAAATTGAAAGCGAGAAATAATCATAAATTATTTTCATTATCGCGATATCCGTAA
241 GTTTGTATCGTAATGGCAGAAGGATTGTCAGCCAATAGACAATGGATAGGACCAGAAGAA
301 GCTGAAGAGTTATTAGATTTTGATATAGCAACACAAATGAGTGAAGAAGGACCACTAAAT
361 CCAGGAGTAAACCCATTTAGGGTACCTGGAATAACAGAAAAAGAAAAGCAAACTACTGT
421 AACATATTACAACCTAAGTTACAAGATCTAAGGAACGAAATTCAAGAGGTTAAACTGGAA
481 GAAGGAAATGCAGGTAAGTTTAGAAGAGCAAGATTTTAAAGGTATTCTGATGAACAAGTA
541 TTGTCCCTGGTTCATGCGTTCATAGGATATTGTATATATTTAGGTAATCGAAATAAGTTA
601 GGATCTTTAAGACATGACATTGATATAGAAGCACCCCAAGAAGAGTGTTATAATAATAGA
661 GAGAAGGGTACAACCTGACAATATAAAATATGGTAGACGATGTTGCCTAGGAACGGTGACT
721 TTGTACCTGATTTTATTTATAGGATTAATAATATATTCACAGACAACCAACGCTCAGGTA
781 GTATGGAGACTTCCACCATTAGTAGTCCCAGTAGAAGAATCAGAAATAATTTTTTGGGAC
841 TGTTGGGCACCAGAAGAACCCGCTGTGAGGACTTTCTTGGGGCAATGATACATCTAAAA
901 GCTAAGACAAATATAAGTATACGAGAGGGACCTACCTTGGGGAATTGGACTAGAGAAATA
961 TGGGCAACATTATTCAAAAAGGCTACTAGACAATGTAGAAGAGGCAGAATATGGAAAAGA
1021 TGGAATGAGACTATAACAGGACCATCAGGATGTGCTAATAACACATGTTATAATGTTTCA
1081 GTAATAGTACCTGATTATCAGTGTTATTTAGATAGAGTAGATACTTGGTTACAAGGGAAA
1141 ATAAATATATCATTATGTCTAACAGGAGGAAAAATGTTGTACAATAAAGTTACAAAACAA
1201 TTAAGCTATTGTACAGACCCATTACAAATCCCCTGATCAATTATACATTTGGACCTAAT
1261 CAAACATGTATGTGGAATACTTCACAAATTCAGGACCCTGAAATACCAAAATGTGGATGG
1321 TGGAATCAAATGGCCTATTATAACAGTTGTAAATGGGAAGAGGCAAAGGTAAAGTTTCAT
1381 TGTCAAAGAACACAGAGTCAGCCTGGATCATGGCGTAGAGCAATCTCGTCATGGAAACAA
1441 AGAAATAGATGGGAGTGGAGACCAGATTTGGAAAGTAAAAAGGTGAAAATATCTCTACAG
1501 TGCAATAGCACAAAAACCTAACCTTTGCAATGAGAAGTTCAGGAGATTATGGAGAAGTA
1561 ACGGGAGCTTGGATAGAGTTTGGATGTCATAGAAATAAATCAAAACATCATTCTGAAGCA
1621 AGGTTTAGAATTAGATGTAGATGGAATGTAGGATCCGATACCTCGCTCATTGATACATGT
1681 GGAAACACTCGAGATGTTTCAGGTGCGAATCCTGTAGATTGTACCATGTATTCAAATAAA
1741 ATGTACAATTGTTCTTTACAAAATGGGTTTACTATGAAGGTAGATGACCTTATTGTGCAT
1801 TTCAATATGACAAAAGCTGTAGAAATGTATAATATTGCTGGAAATTGGTCTTGATACATCT
1861 GACTTGCCATCGTCATGGGGGTATATGAATTGTAATTGTACAAATAGTAGTAGTATAT
1921 AGTGGTACTAAAATGGCATGTCCTAGCAATCGAGGCATCTTAAGGAATTGGTATAACCCA
1981 GTAGCAGGATTACGACAATCCTTAGAACAGTATCAAGTTGTAAAACAACCAGATTACTTA
2041 GTGGTCCCAGAGGAAGTCATGGAATATAAACCTAGAAGGAAAAGGGCAGCTATTCATGTT
2101 ATGTTGGCTCTTGCAACAGTATTATCTATTGTCGGTGCAGGGACGGGGGCTACTGCTATA
2161 GGGATGGTAACCCAATACCACCAAGTTCTGGCAACCCATCAAGAAGCTATAGAAAAGGTG
2221 ACTGAAGCCTTAAAGATAAACAACCTTAAGATTAGTTACATTAGAGCATCAAGTACTAGTA
2281 ATAGGATTAAAAGTAGAAGCTATGGAAAAATCTTATATACAGCTTTCGCTATGCAAGAA
2341 TTAGGATGTAATCAAAATCAATTCTTCTGCAAAATCCCTCCTGGGTTGTGGACAAGGTAT

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FIG. 7 (cont'd)

2401 AATATGACTATAAATCAAACAATATGGAATCATGGAAATATAACTTTGGGGGAATGGTAT
2461 AACCAAACAAAAGATTTACAACAAAAGTTTTATGAAATAATAATGGACATAGAACAAAAT
2521 AATGTACAAGGGAAAACAGGGATACAACAATTACAAAAGTGGGAAGATTGGGTTAGGATGG
2581 ATGGGAAATATTCCACAATATTTAAAGGGACTATTGGGAGGTATCTTGGGAATAGGATTA
2641 GGAGTGTTATTATTGATTTTATGTTTACCTACATTGGTTGATTGTATAAGAAATTGTATC
2701 CACAAGATACTAGGATACACAGTAATTGCAATGCCTGAAGTAGAAGGAGAAGAAATACAA
2761 CCACAAATGGAATTGAGGAGAAATGGTAGGCAATGTGGCATGTCTGAAAAAGAGGAGGAA
2821 TGAATCGATACATCATGCAGTGGTTAAACAAAACATTTTTATTCTCAAATGAGATAAAG
2881 TGAAAATATATATCATTATATTACAAAGTACAATTATTTAGGTTTAATCATGGGGAATGG
2941 ACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGTAGTAATGTTGCTGTAGGAGTAGG
3001 GGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTTCAGATGGGCCATTAGAATGGCTAATGT
3061 ATCTACAGGACGAGAACCTGGTGATATAACCAGAGACTTTAGATCAACTAAGGTTGGTTAT
3121 TTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCTAGCAAAGAAATTGATATGGCAAT
3181 TGTGACATTAAAAGTCTTTGCGGTAGCAGGACTTTTGAATATGACGGTGTCTACTGCTGC
3241 TGCAGCTGAAAATATGTATTCTCAAATGGGATTAGACACTAGGCCATCTATGAAAGAAGC
3301 AGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCTATTCAAACAGTAAATGGAGTACC
3361 ACAATATGTAGCACTTGACCCAAAAATGGTGTCCATTTTCATGGAAAAGGCAAGAGAAGG
3421 ACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCCTTCTCTGCAAATTTAACACCTAC
3481 TGACATGGCCACATTAATAATGGCCGCACCAGGGTGCCTGTCAGATAAAGAAATATTGGA
3541 TGAAAGCTTAAAGCAACTGACAGCAGAAATATGATCGCACACATCCCCCTGATGCTCCCAG
3601 ACCATTACCCTATTTTACTGCAGCAGAAATTATGGGTATAGGATTAAGTCAAGAACAACA
3661 AGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGTAGAGCATGGTATCTCGAGGCATT
3721 AGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGAGCTGTGCAGTTAAGACAAGGAGC
3781 TAAGGAAGATTATTCATCCTTTATAGACAGATTGTTTGCCCAAATAGATCAAGAACAAAA
3841 TACAGCTGAAGTTAAGTTATATTTAAACAGTCATTAAGCATAGCTAATGCTAATGCAGA
3901 CTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCTAGAAGAAAAGTTGAGAGC
3961 TTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAACTCTTGGCAGAAGCTCTTACAAA
4021 AGTTCAAGTAGTGCAATCAAAAGGATCAGGACCAGTGTGTTTAAATTGTAAAAAACAGG
4081 ACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGTAATAAATGTGGAAAACCTGGTCA
4141 TCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAATTCGGGAAACTGGAAGGCGGGCG
4201 AGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTAATGCCATCTGCACCTCCAATGGA
4261 GGAGAACTATTGGATTTATAAATTATAATAAAGTAGGTACGACTACAACATTAGAAAAG
4321 AGGCCAGAAATACTTATATTTGTAAATGGATATCCTATAAAATTTTTATTAGATACAGGA
4381 GCAGATATAACAATTTTAAATAGGAGAGATTTTCAAGTAAAAAATTCTATAGAAAATGGA
4441 AGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAGGAACAAATTATATTAATGTACAT
4501 TTAGAGATTAGAGATGAAAATTATAAGACACAATGTATATTTGGTAATGTTTGTGTCTTA
4561 GAAGATAACTCATTAAATACAACCATTATTGGGGAGAGATAATATGATTAAATTCAATATT
4621 AGGTTAGTAATGGCTCAATAATTTTATCCCGGGTTTTTATAGCTAATTAGTCATTTTTTCG
4681 TAAGTAAGTATTTTTATTTAATACTTTTTATTGTACTTATGTTAAAT

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FIG. 8 Predicted nucleotide sequence of vCP329 insertion. The H6 promoter starts at position 2146. The coding sequence for FIV 97TM is from position 2022 to position 42. The I3L promoter starts at position 2253. The FIV gag start codon is at position 2353 and the pol stop codon is at position 3766.

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1   TTAATCAATAAAAAGAATTCCTGCAGGAATTCATAAAAATCATTCTTCTCCTTCTACTTC
61  AGGCATTGCAATTACTGTGTATCCTAGTATCTTGTGGATACAATTTCTTATACAATCAAC
121 CAATGTAGGTAAACATAAAATCAATAAACAACCTCCTAATCCTATTCCCAAGATACCTCC
181 CAATAGTCCCCTTTTCTTCTAGGTTTATATTCCATGACTTCCTCTGGGACCACTAAGTA
241 ATCTGGTTGTTTTACAACCTTGATACTGTTCTAAGGATTGTCGTAATCCTGCTACTGGGTT
301 ATACCAATTCCTTAAGATGCCTCGATTGCTAGGACATGCCATTTTAGTACCACTATAACT
361 ACTACTACTATTTGTACAATTACAATTCATATACCCCCATGACGATGGCAAGTCAGATGT
421 ACAAGACCAATTTCCAGCAATATTATACATTTCTACAGCTTTTGTGCATATTGAAATGCAC
481 AATAAGGTCATCTACCTTCATAGTAAACCCATTTTGTAAAGAACAATTGTACATTTTATT
541 TGAATACATGGTACAATCTACAGGATTGCGACCTGAAACATCTCGAGTGTTTCCACATGT
601 ATCAATGAGCGAGGTATCGGATCCTACATTCCATCTACATCTAATTCTAAACCTTGCTTC
661 AGAATGATGTTTTGATTTATTTCTATGACATCCAACTCTATCCAAGCTCCCGTTACTTC
721 TCCATAATCTCCTGAACTTCTCATTGCAAAGGTTAGGTTTTTTGTGCTATTGCACTGTAG
781 AGATATTTTTCACCTTTTTTACTTTCCAAATCTGGTCTCCACTCCCATCTATTTCTTTGTTT
841 CCATGACGAGATTGCTCTACGCCATGATCCAGGCTGACTCTGTGTTCTTTGACAATGAAA
901 CTTTACCTTTGCCTCTTCCCATTACAACTGTTATAATAGGCCATTTGATTCCACCATCC
961 ACATTTTGGTATTTTCAGGGTCTGAATTTGTGAAGTATTCACACATACATGTTTGATTAGG
1021 TCCAAATGTATAATTGATCAGTGGGATTTGTAATGGGTCTGTACAATAGCTTAATTGTTT
1081 TGTAACCTTTATTGTACAACATTTTCTCCTGTTAGACATAATGATATATTTATTTTCCC
1141 TTGTAACCAAGTATCTACTCTATCTAAATAACACTGATAATCAGGTACTATTACTGAAAC
1201 ATTATAACATGTGTTATTAGCACATCCTGATGGTCTGTTATAGTCTCATTCCATCTTTT
1261 CCATATTCTGCCTCTTCTACATTGTCTAGTAGCCTTTTTTGAATAATGTTGCCCATATTTT
1321 TCTAGTCCAATTCCCCAAGGTAGGTCCCTCTCGTATACTTATATTTGTCTTAGCTTTTAG
1381 ATGTATCATTGCCCCAAGAAAGTCCTGACAGGCGGGTCTTCTGGTGCCCAACAGTCCCA
1441 AAAAATTATTTCTGATTCTTCTACTGGGACTACTAATGGTGGAAGTCTCCATACTACCTG
1501 AGCGTTGGTTGTCTGTGAATATATTATTAATCCTATAAAATAAAATCAGGTACAAAGTCAC
1561 CGTTCCTAGGCAACATCGTCTACCATATTTTATATTGTCAGTTGTACCCTTCTCTCTATT
1621 ATTATAACACTCTTCTTGGGGTGCTTCTATATCAATGTCATGTCTTAAAGATCCTAACTT
1681 ATTTGATTACCTAAATATATACAATATCCTATGAACGCATGAACCAGGGACAATACTTG
1741 TTCATCAGAATACCTTAAAAATCTTGCTCTTCTAAACTTACCTGCATTTCTTCTTCCAG
1801 TTTTACCTCTTGAATTTTCGTTCTTAGATCTTGTAACCTTAGGTTGTAATATGTTACAGTA
1861 GTTTTGCTTTTCTTTTTCTGTTATTCCAGGTACCCTAAATGGGTTTACTCCTGGATTAG
1921 TGGTCCTTCTTCACTCATTGTTGTTGCTATATCAAATCTAATAACTCTTCAGCTTCTTC
1981 TGGTCCTATCCATTGTCTATTGGCTGCAAATCCTTCTGCCATTACGATACAACTTAACG
2041 GATATCGCGATAATGAAATAATTTATGATTATTTCTCGCTTTCAATTTAACACAACCCTC
2101 AAGAACCTTTGTATTTATTTTCACTTTTAAAGTATAGAATAAAGAACTGCAGCTAATTAA
2161 TTAAGCTACAAATAGTTTCGTTTTTACCTTGTCTAATAACTAATTAATTAAGGATCCCCC
2221 GTACCGGGCCCCCCTCGAGGTGACATCGATACATCATGCAGTGGTTAAACAAAAACAT
2281 TTTTATTCTCAAATGAGATAAAGTGAAAATATATATCATTATATTACAAAGTACAATTAT
2341 TTAGGTTTAATCATGGGGAATGGACAGGGGCGAGATTGGAAAATGGCCATTAAGAGATGT
2401 AGTAATGTTGCTGTAGGAGTAGGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTTACA
2461 TGGGCCATTAGAATGGCTAATGTATCTACAGGACGAGAACCCTGGTGATATACCAGAGACT
2521 TTAGATCAACTAAGGTTGGTTATTTGCGATTTACAAGAAAGAAGAGAAAAATTTGGATCT
2581 AGCAAAGAAATTGATATGGCAATTGTGACATTAAAGTCTTTGCGGTAGCAGGACTTTTG
2641 AATATGACGGTGTCTACTGCTGCTGCAGCTGAAAATATGTATTCTCAAATGGGATTAGAC
2701 ACTAGGCCATCTATGAAAGAAGCAGGTGGAAAAGAGGAAGGCCCTCCACAGGCATATCCT
2761 ATTCAAACAGTAAATGGAGTACCACAATATGTAGCACTTGACCCAAAAATGGTGTCCATT

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FIG. 8 (cont'd)

2821 TTCATGGAAAAGGCAAGAGAAGGACTAGGAGGGGAGGAAGTTCAACTATGGTTTACTGCC
2881 TTCTCTGCAAATTTAACACCTACTGACATGGCCACATTAATAATGGCCGCACCAGGGTGC
2941 GCTGCAGATAAAGAAATATTGGATGAAAGCTTAAAGCAACTGACAGCAGAATATGATCGC
3001 ACACATCCCCCTGATGCTCCCAGACCATTACCCTATTTTACTGCAGCAGAAATTATGGGT
3061 ATAGGATTAACCTCAAGAACAACAAGCAGAAGCAAGATTTGCACCAGCTAGGATGCAGTGT
3121 AGAGCATGGTATCTCGAGGCATTAGGAAAATTGGCTGCCATAAAAGCTAAGTCTCCTCGA
3181 GCTGTGCAGTTAAGACAAGGAGCTAAGGAAGATTATTCATCCTTTATAGACAGATTGTTT
3241 GCCCAAATAGATCAAGAACAAAATACAGCTGAAGTTAAGTTATATTTAAACAGTCATTA
3301 AGCATAGCTAATGCTAATGCAGACTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGT
3361 ACCCTAGAAGAAAAGTTGAGAGCTTGTCAAGAAATAGGCTCACCAGGATATAAAATGCAA
3421 CTCTTGGCAGAAGCTCTTACAAAAGTTCAAGTAGTGCAATCAAAGGATCAGGACCAGTG
3481 TGTTTTAATTGTAAAAAACCCAGGACATCTAGCAAGACAATGTAGAGAAGTGAAAAAATGT
3541 AATAAATGTGGAAAACCTGGTCATCTAGCTGCCAAATGTTGGCAAGGAAATAGAAAGAAT
3601 TCGGGAAACTGGAAGGCGGGGCGAGCTGCAGCCCCAGTGAATCAAATGCAGCAAGCAGTA
3661 ATGCCATCTGCACCTCCAATGGAGGAGAACTATTGGATTTATAAATTATAATAAAGTAG
3721 GTACGACTACAACATTAGAAAAGAGGCCAGAAATACTTATATTTGTAAATGGATATCCTA
3781 TAAAATTTTTTATTAGATACAGGAGCAGATATAACAATTTTAAATAGGAGAGATTTTCAAG
3841 TAAAAAATTCTATAGAAAATGGAAGGCAAAATATGATTGGAGTAGGAGGAGGAAAGAGAG
3901 GAACAAATTATATTAATGTACATTTAGAGATTAGAGATGAAAATTATAAGACACAATGTA
3961 TATTTGGTAATGTTTGTGTCTTAGAAGATAACTCATTATAACAACCATTATTGGGGAGAG
4021 ATAATATGATTAAATTCAATATTAGGTTAGTAATGGCTCAATAATTTTATCCCGGGTTTT
4081 TATAGCTAATTAGTCATTTTTTCGTAAGTAAGTATTTTTTATTTAATACTTTTTTATTGTA
4141 TATGTTAAAT