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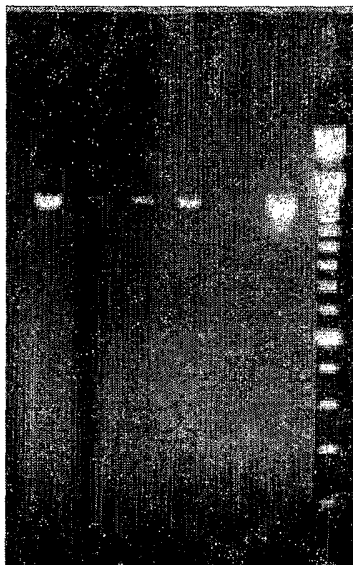
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(54) Title: A METHOD OF DIAGNOSING FIV

FIV subtype

A B C D

neg. control
pos. control
DNA ladder



(57) Abstract: The present invention is directed to a method for diagnosing feline immunodeficiency virus (FIV) infection. The method involves the PCR amplification of a DNA sample obtained from a cat to be diagnosed. The PCR amplification utilizes a first gag-specific DNA primer derived from the gag gene region of an FIV genome and a second FIV-specific DNA primer that binds to a region of an FIV genome suitably spaced from the first primer to permit DNA amplification to occur. The use of such primers advantageously allows for the exponential amplification of proviral DNA of any FIV subtype, thereby providing a universal means to diagnose FIV infection.



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A METHOD OF DIAGNOSING FIV

FIELD OF THE INVENTION

[0001] The present invention relates to a method of diagnosing feline immunodeficiency virus (FIV) infection using a novel polymerase chain reaction (PCR) assay. In particular, the present invention relates to a universal FIV diagnostic method utilizing FIV *gag*-specific primers which permit diagnosis of any FIV infection.

BACKGROUND OF THE INVENTION

[0002] The feline immunodeficiency virus, first identified as a T-lymphotropic virus (Pedersen et al., 1987), is a lentivirus of the family Retroviridae and is related to the human immunodeficiency virus (HIV) (Olmsted et al. 1989a; Olmsted et al., 1989b; Pedersen et al., 1987; Sparkes et al., 1993). Infected cats typically develop CD4+ T lymphocytopenia that leads to immunodeficiency, opportunistic infections, increased occurrence of neoplasia, or a neurological syndrome. Based on genetic, morphological and clinical similarities, FIV has been a valuable model for understanding aspects of HIV pathogenesis and for developing intervening strategies to control infection and disease (Bendinelli et al., 1995; Johnson et al., 1994; Okada et al., 1994; Pedersen et al., 1987).

[0003] Lentiviruses, being retroviruses, invade their host organism by attaching to receptors on the extracellular surface of a host cell, after which the viral RNA genome is introduced into the host cell. Through the enzymatic activity of the viral reverse transcriptase, a DNA copy of the viral RNA is made within the host cell, this DNA copy being able to integrate into the host cell's genome. Reproduction of the virus occurs not only through the activity of the host cell's self-replicative machinery, but also through the co-opting of the host cell's transcription and translation mechanisms in order to produce more of the functional virus within the host. Exponential reproduction of the virus within the host cell thus occurs. Lentiviral structure, in terms of the relative ordering and known function of the various major genes in lentiviral RNA genome has been described in United States Patent No. 5,478,724. Two of the major genes in the lentiviral genome are

gag and *env*, which respectively encode for the structural capsid proteins and viral extra-cellular envelope proteins. Situated at either end of the viral genome and adjacent to the *gag* and *env* genes are the long terminal repeats (LTR's), while situated between *gag* and *env* are the genes encoding for the nucleic acid binding proteins, the viral protease, reverse transcriptase, the tethering protein, RNase H, and the viral endonuclease.

Overall, the lentiviral genome size is approximately 10,000 base pairs, and with respect to FIV, there has been found to be about 30% sequence variation between various viral strains for the *env* gene and about 10% sequence variation for the remainder of the FIV genes. Kakinuma et al. (1995) provide a detailed analysis of the nucleotide and amino acid differences between the North American, European and Japanese strains of FIV.

[0004] FIV is distributed worldwide (Bachmann et al., 1997; Ishida et al., 1989; Nishimura et al., 1998). The prevalence of infection is highly variable ranging from 1 % in cats at low risk in the United States and Canada (Yamamoto et al., 1989) to 44 % in symptomatic cats in Japan (Hohdatsu et al., 1998), depending upon factors such as age, gender and indoor or outdoor housing. Currently, FIV isolates are classified into 5 different subtypes designated as A, B, C, D and E, based on envelope sequence analysis (Nishimura et al., 1998). Subtype A was found in California and Europe while subtype B was prevalent in the Central and Eastern United States (Sodora et al., 1994). Subtype C has been reported from British Columbia and Ontario, Canada (Bachmann et al., 1997; Sodora et al., 1994); while subtypes D and E have been reported from Japan (Hohdatsu et al., 1996; Hohdatsu et al., 1998; Nishimura et al., 1998) and Argentina (Pecoraro et al., 1996), respectively. Classification into subtypes may be accomplished by different methods including subtype-specific PCR (Nishimura et al., 1998), heteroduplex mobility assay (Bachmann et al., 1997), restriction fragment polymorphism (Hohdatsu et al., 1998), and phylogenetic analysis of proviral DNA sequences (Worobey and Holmes, 1999). Although most studies were based on the sequence variation found in the *env* gene, comparison of sequences of the *gag* gene can also be useful, since this gene as well has marked variability among different FIV isolates (Hohdatsu et al., 1998; Kakinuma et al., 1995).

[0005] Reports in the literature indicate that co-infection with two different HIV-1 strains might occur with exposure to a second virus shortly after the initial infection, or after the initial infection has been established, a condition termed superinfection (Jost et al., 2002). This superinfection phenomenon was also experimentally induced with FIV subtypes A and B, both in vitro and in vivo (Okada et al., 1994). In another study, consecutive exposure of cats to two different FIV strains resulted in superinfection in one cat and recombination in another cat (Kyaw-Tanner et al., 1994), but recombination has rarely been documented in FIV under natural conditions (Bachmann et al., 1997). This superinfection phenomenon and the potential for viral recombination will be of significance as the development of vaccines against lentiviruses such as HIV and FIV will require knowledge of the viral variants being transmitted in the target population (Lole et al., 1999). Since genetic diversity among viruses is extensive, it has been debated whether a vaccine should include a single virus strain, a broad spectrum of viral variants, or only those that are relevant to a particular geographical area (Gao et al., 1998). In addition, intersubtype recombination might complicate the already difficult task of developing vaccines effective against multiple subtypes (Siepel, 1995). Similar to HIV, the high genetic variability displayed by FIV strains, and the possibility of recombination in geographical areas where more than one subtype is present, are major considerations for developing an effective vaccine. Thus, greater understanding of the genetic diversity of FIV would provide a better rationale for vaccine design, and elucidate mechanisms of retroviral recombination.

[0006] Recently, a vaccine against FIV was introduced onto the marketplace (Connell, 2003), however, the general use of the vaccine imposes problems with respect to accurately diagnosing FIV infection cats. Standard assays for detecting the presence of FIV in cats involve the use of antibody detection methods, such as the ELISA technique, which allow for the detection of antibodies generated by the infected cat during its immunological response to viral infection. Problematic, however, is the fact that for FIV-vaccinated cats, an antibody detection assay will generate a false-positive result as these cats will a priori have a high antibody titer due to the vaccination.

[0007] In view of the foregoing, it is clear that there is the need to provide a diagnostic method that allows for detection of infection by any FIV subtype in a cat. It is also desirable to provide a method that allows for the diagnosis of cats that are truly FIV-infected versus those uninfected cats that have previously been vaccinated and thus have circulating anti-FIV antibodies. Furthermore, it is also desirable to provide an FIV diagnostic method with sufficient sensitivity to allow for the diagnosis of FIV infection during the period between initial infection and the generation of an immunological response in the infected cat.

SUMMARY OF THE INVENTION

[0008] Accordingly, in one aspect, the present invention provides a method for diagnosing FIV infection in a cat comprising the steps of:

- (i) obtaining a DNA-containing sample from the cat;
- (ii) extracting the DNA from the sample;
- (iii) subjecting the extracted DNA to PCR amplification to form an FIV-specific amplicon, wherein the amplification utilizes a first *gag*-specific primer which binds to a first region on an FIV genome and a second FIV-specific primer which binds to a second region on an FIV genome, and wherein the first and second regions are spaced such that amplification can occur; and
- (iv) determining whether an FIV-specific amplicon is generated during step (iii), wherein the presence of an FIV-specific amplicon is indicative of an FIV infection in a cat.

[0009] In another aspect of the present invention, a kit is provided comprising a first FIV *gag*-specific primer which binds to a first region on an FIV genome and a second FIV-specific primer which binds to a second region on an FIV genome, and wherein the first and second regions are spaced such that amplification can occur.

[0010] In another aspect of the present invention, an FIV *gag*-specific primer is provided selected from the group consisting of: a primer comprising the sequence 5'-

AGATACCATGCTCTACAC TGC-3' (SEQ ID No: 4), a primer comprising the sequence 5'-GGTATATCACCAGGTTCTGCT-3' (SEQ ID No: 6) and a functionally equivalent primer comprising a nucleotide sequence which exhibits at least about 80% sequence homology with one of SEQ ID No: 4 or SEQ ID No: 6.

[0011] These and other aspects of the present invention are described by reference to the following figures in which:

BRIEF DESCRIPTION OF THE FIGURES

[0012] Figure 1 illustrates the nucleotide sequence (SEQ ID NO: 1) of the FIV Petaluma strain (GenBank Accession No. M25381);

[0013] Figure 2 illustrates the upstream or downstream positioning of various primers relative to the consistent breakpoint identified within the FIV *gag* gene region;

[0014] Figure 3 is an electrophoretic gel evidencing PCR amplification of FIV proviral DNA of various subtypes using an FIV *gag*-specific primer, Gag1-r, and an FIV-specific primer, LTR1-f;

[0015] Figure 4 is an electrophoretic gel evidencing PCR amplification of FIV proviral DNA of various subtypes using an FIV *gag*-specific primer, Gag 781-r, and an FIV-specific primer, Gag 635-f ;

[0016] Figure 5 illustrates the positioning of LTR1-f, LTR2-f , Gag1-r, Gag635f and Gag718r primers relative to the FIV Petaluma genome.

DETAILED DESCRIPTION OF THE INVENTION

[0017] The present invention is directed to a method of diagnosing any feline immunodeficiency virus (FIV) infection in a cat through the use of a polymerase chain reaction (PCR) assay. DNA from a DNA-containing sample obtained from a cat to be diagnosed is subjected to PCR utilizing a first FIV *gag*-specific primer and a second FIV-

specific primer to form an FIV-specific amplicon. The detection of an FIV-specific amplicon following the PCR amplification indicates FIV infection in the subject cat. The use of an FIV *gag*-specific primer advantageously allows for the exponential amplification of proviral DNA of any FIV subtype, thereby providing a universal means to diagnose FIV infection.

[0018] As used herein, the term “FIV” means feline immunodeficiency virus including all strains, such as the Petaluma strain and strain USgaB01; all subtypes, such as subtypes A through E, and evolving subtypes B such as FIV Texas (Weaver et al, 2004); isolates including isolates from Asia (Kakinuma et al. 1995), Europe (Pistello et al., 1997), Australia (Greene et al., 1993) and North America; and variants and recombinants thereof.

[0019] As used herein, the term “viral subtype ” refers generally to FIV genomes that have greater than 80% similarity to each other and exhibit at least 20% divergence within the FIV DNA sequence of the *env* gene from other clusters, including for example FIV subtypes A through E.

[0020] The term "primer" as used herein is meant to encompass a single-stranded DNA fragment that is capable of binding to an FIV genomic template to initiate the formation of an amplicon in the PCR amplification process. Such PCR primers are typically about 10 to 35 nucleotide bases in length; however, primers outside of this size range may also be used as one of skill in the art will appreciate.

[0021] A primer “derived” from a particular FIV DNA sequence or region of the FIV genome, i.e. primer region, refers to the fact that the primer may have a forward or reverse complementary sequence to the DNA sequence on the strand of DNA upon which the primer binds, and may also vary from the native sequence by nucleotide substitutions, deletions or insertions which do not affect its function as a primer for use in PCR.

[0022] As used herein, the term “amplicon” refers to the DNA product that results from a polymerase chain reaction (PCR). The amplicon is defined by the primers which are designed to allow for the amplification of a particular DNA product. The amplicon, thus, is a DNA product which includes the primer regions, i.e. the regions to which each of the PCR primers bind, as well as the sequence between the primer regions.

[0023] To conduct the diagnostic method of the present invention, a DNA-containing biological sample is obtained from a cat to be diagnosed. Appropriate DNA-containing biological samples for use in the present method include, but are not limited to, saliva, urine, semen and other bodily secretions, as well as hair, epithelial cells and the like. Although such non-invasively obtained biological samples are preferred for use in the present method, one of skill in the art will appreciate that invasively-obtained DNA-containing biological samples, may also be used in the present method, including for example, blood, serum, bone marrow, cerebrospinal fluid (CSF) and tissue biopsies such as lymph node samples. Techniques for the invasive process of obtaining such samples are known to those of skill in the art.

[0024] It may be necessary, or preferable, to extract the DNA from the biological sample prior to proceeding with the PCR amplification. Methods of DNA extraction are well-known to those of skill in the art and include chemical extraction techniques utilizing phenol-chloroform (Sambrook et al., 1989), guanidine-containing solutions, or CTAB-containing buffers. As well, as a matter of convenience, commercial DNA extraction kits are also widely available from laboratory reagent supply companies, including for example, the QIAamp DNA Blood Minikit available from QIAGEN (Chatsworth, CA), or the Extract-N-Amp blood kit available from Sigma (St. Louis, MO).

[0025] Once an appropriate DNA sample is obtained, it is subjected to PCR amplification using primers derived from FIV in order to determine whether or not FIV DNA is present in the sample, and thus, whether there is FIV infection in the subject cat. DNA amplification by PCR is a technique that is readily known by those of skill in the art, and described in United States Patent No. 4,683,195. Generally, PCR allows for the amplification of a DNA template strand, the termini of which are defined by a forward and reverse primer pair, by the repeated polymerase-assisted synthesis of the DNA strand. PCR conditions may vary depending on the length and nucleotide sequence of the primer. For example, an annealing temperature of less than 50 °C may be required when one, or both, of the PCR primers is less than 20 nucleotides in length or contains a low percentage of guanine and/or cytosine bases. Preferably, an annealing temperature of 50 to 72 °C, and more preferably 55 to 65 °C is used for PCR. As well, the concentration of MgCl₂ utilized in the PCR buffer may vary depending on the nucleotide base

composition and length of the PCR primers. The concentration of MgCl_2 utilized may be in the range of 0.5 to 3.0 mM; however, a more preferable concentration range is that of 1.0 to 2.5 mM, and most preferable is a concentration range of 1.4 to 2.0 mM. The length of time allowed for the thermostable DNA polymerase to extend the daughter strand of DNA will vary depending on the nucleotide length of the amplicon. Generally, an extension time in the range of 45 to 120 seconds for every one thousand nucleotide bases of the amplicon is appropriate; however, an extension time of 50 to 100 seconds is preferred, and an extension time of 60 to 90 seconds is most preferred.

[0026] To diagnose FIV infection in a cat using the present PCR method, a first FIV *gag*-specific PCR primer is utilized. The term “*gag*-specific” as used herein is meant to denote a primer that is specific to the *gag* region of an FIV genome, for example, a primer that exhibits at least about 80% sequence homology with an FIV *gag* sequence and retains primer function. Preferably the primer exhibits at least about 90% sequence homology with an FIV *gag* sequence, and most preferably, the primer exhibits at least 95% sequence homology with an FIV *gag* sequence. The term “*gag*” refers to the *gag* gene region of an FIV genome which encodes structural capsid proteins. In the Petaluma FIV strain, for example, the *gag* region is defined by nucleotides 628 to 1980 of the genome. However, as will be appreciated by one of skill in the art, the exact nucleotide location of this region may vary somewhat among FIV strains and subtypes. For use in the present method, the *gag*-specific primer is derived from a region of the *gag* gene that is highly conserved among FIV, e.g. a region exhibiting at least 95% sequence homology among FIV. In one embodiment of the present invention, as described in more detail in the specific examples herein, the *gag*-specific primer is derived from the 5' end of the *gag* gene, for example, the region spanning nucleotides 628 to 1000. Preferably, the *gag*-specific primer is derived from the *gag* nucleotide region 700 to 800, and more preferably, from the *gag* nucleotide region 768 to 788. In another embodiment, the *gag*-specific primer is derived from the central region of the *gag* gene, for example, the region spanning nucleotides 1200 to 1500. Preferably, the *gag*-specific primer is derived from the *gag* nucleotide region 1350 to 1450, and more preferably, from the *gag* nucleotide region 1409 to 1389. Moreover, the *gag*-specific primer is also defined by a GC content between about 45% to 60% and a melting temperature (T_m) between about

50 °C to 70 °C and possesses no variation in at least the three nucleotides at the 3' end of the primer from the FIV *gag* gene sequence.

[0027] The second PCR primer of the required PCR primer pair used in the present method is an FIV-specific primer. The term "FIV-specific" as used herein is meant to denote a primer that is specific to FIV, i.e. exhibiting suitable sequence homology with a region in the FIV genomic template such that the primer readily binds to the FIV template under standard PCR conditions, for example a sequence homology of at least about 80% with a region in an FIV genome, and more preferably at least about 90% sequence homology with a region of the FIV genome, but is not derived from any particular region of the FIV genome. It is important, however, that the FIV-specific primer be suitable to function effectively as a second primer in the present PCR method, i.e. the primer binds to a region of an FIV genome suitable for PCR amplification to occur, for example to a region which is appropriately spaced from the binding region of the *gag*-specific primer to allow for amplification to occur. In this regard, it is preferable that the second primer be directed to a region that is within about 1500 nucleotides from the binding region of the FIV *gag*-specific primer. The FIV-specific primer may, for example, be derived from the 5'LTR or long terminal repeat region of the FIV genome such as the region defined by nucleotides 1 to 355 inclusive of an FIV genome. Specific examples of suitable primers derived from this LTR region include, but are not limited to, primers derived from nucleotides 122-141 of the LTR and primers derived from nucleotides 285-309 of the LTR. The FIV-specific primer may also be derived from within the *gag* region of the FIV genome, within nucleotides 628 to 1980 of the FIV genome. Specific examples of suitable FIV-specific primers derived from the *gag* region include primers derived from nucleotides 625 to 643 of FIV *gag*. As set out above, the FIV-specific primer may be based on or derived from a native FIV sequence, but may include some nucleotide variation such as nucleotide insertion, deletion or substitution which does not affect its performance as a primer.

[0028] The FIV primers used in the present PCR method are produced using methods of DNA synthesis well known to those of skill in the art, including for example, the phosphotriester or phosphodiester methods described in U.S. Pat. No. 4,683,195, the contents of which are incorporated herein by reference, either manually or through the

use of an automated DNA synthesizing apparatus, such as an ABI 3900 High-Throughput DNA Synthesizer as manufactured by Applied Biosystems.

[0029] Given the availability of FIV nucleotide sequence information, as set out in Figure 1 herein, FIV *gag*-specific and FIV-specific primers for use in the present PCR method may be designed. Publicly available sequence databases, such as GenBank (<http://www.ncbi.nlm.nih.gov/>), permit access to sequences of other FIV strains or subtypes. The conservation of such FIV genome sequences can readily be established through the use of various web-based sequence alignment tools, such as the CLUSTAL W Multiple Sequence Alignment (<http://clustalw.genome.ad.jp/>), as would be appreciated by one of skill in the art.

[0030] Once the PCR amplification is completed, it is necessary to determine whether or not a targeted FIV-specific amplicon has been produced. The term "targeted" refers to an FIV-specific amplicon that should have resulted from the PCR amplification using the selected *gag*-specific and FIV-specific primers if FIV DNA was present in the sample, i.e. if the cat from which the sample was obtained was infected by FIV. This can be accomplished by various methods that are known to those of skill in the art, such as by electrophoresis and Southern blot analysis (see Sambrook et al., 1989; Southern, 1975). For example, amplicon determination may be conducted by subjecting a portion of the PCR reaction product to analysis by electrophoresis against known DNA size markers. Southern blot analysis of the electrophoresed sample is then conducted to determine if the desired FIV-specific amplicon resulted from the PCR amplification process by hybridization to known DNA sequences.

[0031] A diagnosis of FIV is made when it is determined that the PCR produced the targeted FIV-specific amplicon. The production of the amplicon is a clear indication that FIV DNA is present in the DNA sample extracted from the cat, and thus, evidence of FIV infection. The absence of the targeted FIV-specific amplicon is a clear indication that FIV DNA is not present in the DNA sample, and thus, that there is no FIV infection. The present method is used to directly determine the presence of FIV DNA as opposed to methods which indirectly detect immunological products of FIV infection, e.g. antibodies. As a result, the present method advantageously precludes false positive

results which readily occur in diagnostic methods which utilize antibody determination. For example, due to the availability of optional FIV vaccination, a false positive result can occur when immunological FIV diagnostic tests are used to diagnose vaccinated cats because the presence of FIV antibodies in a vaccinated cat is not necessarily indicative of FIV infection.

[0032] In one embodiment of the present invention, DNA extracted from whole blood samples obtained from FIV-infected cats were assayed using the present PCR method. As described in detail in the specific examples that follow, the FIV *gag*-specific primer used in the PCR was derived from nucleotides 1409 to 1389 (i.e. the reverse complementary sequence to nucleotides 1389 to 1409) of the *gag* region having the following nucleotide sequence: 5'-AGATACCATGCTCTACACTGC-3' (SEQ ID No: 4). The FIV-specific primers used were derived from the LTR region, nucleotides 122-141 and 285-309, having the following specific sequences, respectively: 5'-TTA ACCGCAAAACCACATCC-3' (SEQ ID No: 2) and 5'-TGAACCCTGTCGTGTA TCTGTGTAA-3' (SEQ ID No: 3). As set out above, and as one of skill in the art will appreciate, these primer sequences are exemplary only. *Gag*-specific primers which exhibit at least 80% sequence homology with SEQ ID No: 4 and are functionally equivalent, i.e. retain primer function, may also be used, as well as functionally equivalent primers derived from other nucleotide regions of *gag* which are highly conserved in all FIV. FIV-specific primers which exhibit at least about 80% sequence homology with either SEQ ID No: 2 or 3 and are functionally equivalent thereto, as well as FIV-specific primers derived from other nucleotide regions of the LTR and surrounding gene regions may similarly be used as long as the FIV-specific primer is derived from a region which is spaced suitably from the *gag*-specific primer in order to permit PCR to occur. Sequence modifications which may be made to the specific exemplified primers to yield functionally equivalent primers without significant loss of function include nucleotide base substitution, deletion and insertion, within an acceptable range of sequence modification. With respect to the *gag*-specific primer, functional equivalents include primers which exhibit at least about 80% sequence homology with a *gag* region that is highly conserved in all FIV, preferably 90% sequence homology and more preferably 95% sequence homology, while retaining a GC content of between about

45% to 60%, a melting temperature of between about 50 °C to 70 °C, and having no variation in the four nucleotides at the 3'-most end of the primer from those in the native FIV *gag* sequence. Accordingly, examples of a functionally equivalent *gag*-specific primer include those which have up to 4 nucleotide substitutions within the 18 nucleotides situated proximal to the 5' end of the primer identified as SEQ ID No: 4, which do not result in the formation of a structure that might inhibit the function of the primer (e.g. a hairpin structure), while retaining the GC content and melting temperature indicated above.

[0033] In another embodiment of the present invention, DNA extracted from whole blood samples obtained from FIV-infected cats were assayed using the present PCR method. As described in detail in the specific examples that follow, the FIV *gag*-specific primer used in the PCR was derived from nucleotides 788-768 (i.e. the reverse complementary sequence to nucleotides 768-788) of the *gag* region having the following nucleotide sequence: 5'-GGTATATCACCAGGTTCTGCT-3' (SEQ ID No: 6). The FIV-specific primer used was also derived from the *gag* region, nucleotides 625-643, having the following specific sequence: 5'-AACATGGGGAATGGACAGG-3' (SEQ ID No: 5).

[0034] In a further aspect of the present invention a kit is provided comprising a first FIV *gag*-specific primer which binds to a first region on an FIV genome and a second FIV-specific primer which binds to a second region on an FIV genome, and wherein the first and second regions are spaced such that amplification can occur. The kit may consist of a variety of primer combinations derived from any FIV genome that allow for the diagnosis of FIV infection in a cat. The first FIV *gag*-specific primer is derived from the FIV *gag* region, generally nucleotides 628-1980 in the FIV genome. The second FIV-specific primer is not particularly restricted with respect to the FIV gene region from which it is derived; however, as set out above, it is important that the FIV-specific primer be derived from a region of an FIV genome that is appropriately spaced from the binding region of the *gag*-specific primer to allow for amplification to occur.

[0035] In a further aspect of the present invention, an FIV *gag*-specific primer is provided. The FIV *gag*-specific primer may be selected from the group consisting of: a

primer comprising the sequence 5'-AGATACCATGCTCTACAC TGC-3' (SEQ ID No: 4), a primer comprising the sequence 5'-GGTATATCACCAGGTTCTGCT-3' (SEQ ID No: 6) and functionally equivalent variant primers comprising a nucleotide sequence which exhibits at least about 95% sequence homology to one of SEQ ID No: 4 or SEQ ID No: 6. Functionally equivalent variants should retain a GC content of between about 45% to 60%, a melting temperature of between about 50 °C to 70 °C, and have no variation in the three nucleotides at the 3' end of the primer from those in the native FIV *gag* sequence. Functional variants preferably comprise at least the nucleotides – CACTGC- at the 3' end of the primer.

[0036] Embodiments of the invention will now be described by reference to the following specific examples which are not to be construed as limiting.

SPECIFIC EXAMPLES

Example 1 – Proviral Amplification of FIV LTR-*gag* region

[0037] The amplification of proviral DNA from the LTR-*gag* gene region from four different subtypes of FIV was accomplished using a primer combination wherein the forward FIV-specific primer (LTR1-f, SEQ ID No: 2) was able to recognize a highly conserved sequence within the LTR region of FIV and the reverse *gag*-specific primer (Gag1-r, SEQ ID No: 4) was able to recognize a highly conserved sequence within the *gag* gene region. Figure 5 illustrates the relative positioning of these primers relative to the FIV genome.

[0038] Materials. Reference FIV subtype A, strain Petaluma (clone 34TF10), was obtained from J. Elder, through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. Subtype C was obtained from J. Mullins, University of Washington, Seattle, WA; and subtype D, strain Shizuoka, by T. Hohdatsu, Kitasato University, Japan. Of the 29 unknown samples, 4 originated from the Eastern United States, 1 from British Columbia, and 24 from Ontario. All feline blood samples that were from Canada and the United States are as set out in Table 1 below. The blood samples were confirmed positive for FIV antibodies by enzyme-linked immunosorbent assay (ELISA) (Pet Check ELISA; IDEXX, Portland, ME) and yielded an FIV-specific

amplicon through PCR amplification. Background information on the samples is provided in Table 1. Base pair identification of all sequences including primers and amplicons is in reference to FIV subtype A, strain Petaluma (GenBank Accession No. M25381) (Talbot et al., 1989).

Table 1 Sample description and subtype distribution of 29 FN primary isolates^a

Sample	Country	Province City /State	Age	Sex	Clinical findings	Subtype
CaONA01	Canada	ON Mount Hope	10	FN	Anemia, cutaneous ulceration	A
CaONA02	Canada	ON Churchill	8	MN	Hyperglobuhemia, lymphopenta, cardiomyopathy	A
CaONA03	Canada	ON Guelph	4	M	Proliferative polyarthritis, lymphopenia	A
CaONA04	Canada	ON Scarborough	5	FN	Severe stomatitis	A
CaONA05	Canada	ON Oakville	8	MN	Severe stomatitis, diabetes mellitus	A
CaONA06	Canada	ON Kincardine	9	MN	Demodecosis, hyperglobulinemia, lymphopenia	A
CaONA07	Canada	ON Guelph	10	MN	Stomatitis, diarrhea	A
CaONA08	Canada	ON Toronto	6	MN	Anaplastic extranodal lymphoma	A
CaONA09	Canada	ON Guelph	9	MN	Chronic diarrhea, stomantis	A
CaONA10	Canada	ON Guelph	NA	MN	Pneumonia, cutaneous ulceration, lymphopenia	A
CaONA11	Canada	ON Guelph	7	MN	Stomatitis, diarrhea	A
CaONA12	Canada	ON Toronto	2	MN	Stomatitis, neutropenia	A
CaONA13	Canada	ON Toronto	4	M	Vomiting, cutaneous ulceration	A
CaONA14	Canada	ON Toronto	NA	F	Clinically healthy	A
CaONA15	Canada	ON Toronto	NA	NA	Gingivitis, diarrhea, multiple cutaneous abscesses	A
CaONA16	Canada	ON Toronto	2	M	NA	A
CaONA17	Canada	ON Ottawa	6	MN	Diarrhea, neutropenia	A
CaONAB01	Canada	ON Toronto	5	FN	Chronic renal disease	A/B
CaONAB02	Canada	ON Brampton	5	MN	Multiple cutaneous abscesses	A/B
CaCINAB03	Canada	ON Kitchener	NA	NA	Anemia, lymphocytosis	A/B
CaONB01	Canada	ON Guelph	7	MN	Uveitis	B
CaONB02	Canada	ON Guelph	10	F	Encephalopathy, hyperglobulinemia	B
CaONB03	Canada	ON Barrie	6	MN	Gingivitis, abscessation, diarrhea	B
CaONB04	Canada	ON Toronto	NA	NA	NA	B
USctB01	USA	CT Plainville	NA	M	Gingivitis, diarrhea	B
USgaB01	USA	GA Athens	8	MN	Seizures, lymphopenia	B
USgaB02	USA	GA Athens	8	MN	Stomatitis, diarrhea	B
USgaB03	USA	GA Athens	5	MN	Lymphadenopathy	B
CaBC01	Canada	BC Vancouver	8	MN	Stomatitis	C

^aAbbreviations: ON, Ontario; FN, female neutered; MN, male neutered; CT, Connecticut; GA, Georgia; BC, British Columbia; NA, not available.

[0039] DNA isolation. DNA was extracted from 200 μ l of each whole blood sample using the QIAamp DNA Blood Minikit (QIAGEN, Chatsworth, CA) following the directions of the manufacturer. The DNA was eluted into 100 μ l of buffer and stored at -20°C.

[0040] LTR-gag PCR. Primers were designed to amplify the LTR-gag region of proviral DNA. First round reactions produced amplicons of 1287 bp using primers LTR1-f (SEQ ID No: 2) and Gag1-r (SEQ ID No: 4) (Table 2). Utilization of a different forward primer in the PCR, namely LTR2-f (SEQ ID No:3), along with the Gag1-r primer (SEQ ID No: 4), resulted in an FIV-specific amplicon of 1127 bp. The primer

sequences are set out in Table 2 below. This DNA fragment included the LTR regions R and U5, the *gag* region coding for the p15 protein and 421 bp of the *gag* region coding for the p24 protein. Amplifications were performed in 25 μ l total volume with 1.25 units of Taq DNA polymerase (Life Technologies, Burlington, ON, Canada), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 μ M of each primer and 2 μ l of purified DNA. If an insufficient amount of an FIV-specific amplicon was generated during an initial PCR reaction, 2 μ l of the particular initial PCR reaction product was used as a template for a subsequent PCR re-amplification treatment. Reactions were cycled 35 times with denaturation at 94°C for 45 sec, annealing at 60°C for 20 sec, and elongation at 72°C for 90 sec. Conditions for a PCR re-amplification treatment were identical as for the initial PCR amplification except the annealing temperature was 64°C for 20 sec.

Table 2. Primer Sequence and Location.

SEQ ID No.	Primer	Sequence (5' → 3')	Location (bp) ^a
SEQ ID No: 2	LTR1-f	TTAACCGCAAAACCACATCC	122-141
SEQ ID No: 3	LTR2-f	TGAACCTGTCGTGTATCTGTGTAA	285-309
SEQ ID No: 4	Gag1-r	AGATACCATGCTCTACACTGC	1409-1389

^a All positions are in reference to subtype A Petaluma (GenBank Accession No. M25381)

[0041] Results. PCR products were identified by electrophoresis in 1% agarose gels and ethidium bromide staining. The results confirm that the present primers, designed to encompass the LTR-*gag* region, consistently amplified proviral DNA of subtypes A, B, C and D. Results using the Gag1-r and LTR1-f primers are illustrated in Fig. 3 in which there is evidence that the 1287 bp amplicon is produced. Reference strains Petaluma (A), FIV-C (C) and Shizuoka (D), as well as 27 of the 29 primary isolates were efficiently amplified after an initial round of PCR; while 2 of 29 samples required a PCR re-amplification treatment in order to allow for amplicon detection. These results demonstrate that this was a highly efficient amplification protocol for detection of proviral FIV DNA. Although subtype E DNA samples were not available, LTR1-f (SEQ ID No: 2) and Gag1-r (SEQ ID No: 4) primers were 100% homologous to corresponding regions in the 5 known FIV subtypes, including subtype E, and, thus, are expected to amplify proviral DNA of subtype E as well.

[0042] Sample preparation for sequencing. Amplicons of the appropriate size were cut out of agarose gels with sterile blades, and the DNA was extracted from the agarose gel using a QIAquick Gel Extraction kit (QIAGEN, Chatsworth, CA). The concentration of purified DNA samples was determined by fluorimetry (PicoGreen, Roche, Montreal, PQ) and adjusted to 30 ng/ μ l. Sequencing primers were the same as for PCR amplification. Amplicon sequences were determined/confirmed by the BigDye Terminator method on an ABI Prism 377 XL DNA Sequencer (DNA Sequencing Facility, Robarts Research Institute, London, ON).

Example 2 – Proviral Amplification of FIV *gag* region

[0043] Amplification of proviral DNA from the *gag* gene region FIV subtypes A, B, C and D (as identified in Example 2) was accomplished using a primer combination in which both the forward and reverse primers were able to recognize conserved sequences within the *gag* region of the FIV genome. The forward FIV-specific primer (Gag 635 f) had a nucleotide sequence as follows: AACATGGGGAATGGACAGG (SEQ ID No: 5) and was derived from nucleotide positions 625 to 643 of the *gag* gene. This represents nucleotide positions 625 to 643 of the FIV genome as shown in Figure 5. The reverse FIV *gag*-specific primer (Gag 781 r) had the nucleotide sequence: GGTATATCACCAGGTTCTGCT (SEQ ID No: 6) and was derived from nucleotide positions 788 to 768 of the *gag* gene. This represent nucleotide positions 768 to 788 of the FIV genome as shown in Figure 5.

[0044] First round PCR reactions using these *gag* primers produced amplicons of 146 bp using primers. Amplifications were performed in 25 μ l total volume with 1.25 units of Taq DNA polymerase (Life Technologies, Burlington, ON, Canada), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.5 μ M of each primer and 2 μ l of purified DNA. If an insufficient amount of an FIV-specific amplicon was generated during an initial PCR reaction, 2 μ l of the particular initial PCR reaction product was used as a template for a subsequent PCR re-amplification treatment under the same conditions. Reactions were cycled 35 times with denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and elongation at 72°C for 30 sec.

[0045] Results. PCR products were identified by electrophoresis in 1% agarose gels and ethidium bromide staining. Results from these proviral amplification experiments are illustrated in Fig. 4 and confirm that *gag*-specific primers consistently amplified proviral DNA of subtypes A, B, C and D. These results demonstrate that this was a highly efficient amplification protocol for universal detection of proviral FIV DNA.

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All references referred to herein are hereby incorporated by reference.

CLAIMS

1. A method for diagnosing FIV infection in a cat comprising the steps of:
 - a. obtaining a DNA-containing sample from the cat,
 - b. extracting the DNA from the sample,
 - c. subjecting the extracted DNA to PCR amplification to form an FIV-specific amplicon, wherein the amplification utilizes a first *gag*-specific primer which binds to a first region on an FIV genome and a second FIV-specific primer which binds to a second region on an FIV genome, and wherein the first and second regions are spaced such that amplification can occur; and
 - d. determining whether an FIV-specific amplicon is generated during step (iii), wherein the presence of an FIV-specific amplicon is indicative of an FIV infection in a cat.
2. A method as claimed in claim 1, wherein the second primer binds to a region in an FIV genome within no more than about 1500 nucleotides of the first primer.
3. A method as claimed in claim 1, wherein the FIV *gag*-specific primer is derived from nucleotides 1389-1409 of an FIV genome.
4. A method as claimed in claim 1, wherein the first primer comprises a sequence selected from the group consisting of: 5'-AGATACCATGCTCTACTGC-3' (SEQ ID No: 4) and functional equivalent sequences thereof which are at least 80% homologous thereto.
5. A method as claimed in claim 1, wherein the first primer has a GC content between about 45% to 60%, a melting temperature (T_m) between about 50 °C to 70 °C and possesses no variation in at least the four nucleotides at the 3'-most end of the primer identified in SEQ ID No: 4.
6. A method as claimed in claim 1, wherein the first primer comprises the sequence: 5'-AGATACCATGCTCTACTGC-3' (SEQ ID No: 4).
7. A method as claimed in claim 1, wherein the second primer is derived from the long terminal repeat (LTR) region of the FIV genome.
8. A method as claimed in claim 7, wherein the second primer is selected from the group consisting of: a primer comprising the sequence 5'-TTAACCGCAAACACATCC-3' (SEQ ID No: 2); a primer comprising the sequence 5'-TGAACCCTGTCGTGTATCTGTGTAA-3' (SEQ ID No: 3) and a functionally equivalent primer comprising a sequence which is at least 80% homologous with one of SEQ ID No: 2 and SEQ ID No:3.

9. A method as defined in claim 8, wherein the second primer is selected from the group consisting of: a primer comprising the sequence 5'-TTAACCGCAAAACCACATCC-3' (SEQ ID No: 2) and a primer comprising the sequence 5'-TGAACCCTGTCGTGTATCTGTGTAA-3' (SEQ ID No: 3).
10. A method as defined in claim 1, wherein the first and second primers are derived from the *gag* region of the FIV genome.
11. A method as defined in claim 1, wherein the second primer comprises a nucleotide sequence selected from the group consisting of: 5'-AACATGGGGAATGGACAGG-3' (SEQ ID No: 5) and a nucleotide sequence which exhibits at least about 80% sequence homology thereto.
12. A method as defined in claim 1, wherein the first primer comprises a nucleotide sequence selected from the group consisting of: 5'-GGTATATCACCAGGTTCTCGT-3' (SEQ ID No: 6) and a nucleotide sequence which exhibits at least about 80% sequence homology thereto.
13. A kit for diagnosing FIV infection in cats comprising a first FIV *gag*-specific primer which binds to a first region on an FIV genome and a second FIV-specific primer which binds to a second region on an FIV genome, and wherein the first and second regions are spaced such that amplification can occur.
14. A kit as claimed in claim 13, wherein the first FIV *gag*-specific primer is derived from nucleotides 1389-1409 of an FIV genome.
15. A kit as claimed in claim 13, wherein the first FIV *gag*-specific primer comprises a sequence selected from the group consisting of: a primer comprising the sequence 5'-AGATACCATGCTCTACAC TGC-3' (SEQ ID No: 4) and a functionally equivalent primer comprising a nucleotide sequence which exhibits at least about 80% sequence homology with SEQ ID No: 4.
16. A kit as claimed in claim 13, wherein the second FIV-specific primer is selected from the group consisting of:
 - a primer comprising a nucleotide sequence 5'-TTAACCGCAAAACCACATCC-3' (SEQ ID No: 2), a primer comprising a nucleotide sequence 5'-TGAACCCTGTCGTGTATCTGTGTAA-3' (SEQ ID No: 3), a primer comprising a nucleotide sequence AACATGGGGAATGGACAGG-3' (SEQ ID No: 5) and a functionally equivalent variant thereof comprising a nucleotide sequence which exhibits at least about 80% sequence homology to any one of SEQ ID No: 2, SEQ ID No:3 and SEQ ID No: 5.
17. A kit as claimed in claim 13, wherein the first FIV *gag*-specific primer comprises the sequence 5'-AGATACCATGCTCTACAC TGC-3' (SEQ ID No: 4).

18. A kit as claimed in claim 17, wherein the second FIV-specific primer comprises a sequence selected from the group consisting of 5'-TTAACCGCAAAACCACATCC-3' (SEQ ID No: 2) and 5'-TGAACCCTGTCGTGTATCTGTGTAA-3' (SEQ ID No: 3).
19. An FIV *gag*-specific primer selected from the group consisting of: a primer comprising the sequence 5'-AGATACCATGCTCTACACTGC-3' (SEQ ID No: 4) and a primer comprising the sequence GGTATATCACCAGGTTCTGCT (SEQ ID No: 6).
20. An FIV-specific primer selected from the group consisting of: a primer comprising a nucleotide sequence 5'-TTAACCGCAAAACCACATCC-3' (SEQ ID No: 2), a primer comprising a nucleotide sequence 5'-TGAACCCTGTCGTGTATCTGTGTAA-3' (SEQ ID No: 3) and a primer comprising a nucleotide sequence AACATGGGGAATGGACAGG-3' (SEQ ID No: 5).

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1  tgggatgagt attggaaccc tgaagaaata gaaagaatgc ttatggacta gggactgttt
61 acgaacaaat gataaaaagga aatagctgag catgactcat agttaagcg ctagcagctg
121 cctaaccgca aaaccacatc ctatggaaag cttgctaata acgtataagt tgttccattg
181 taagagtata taaccagtgc tttgtgaaac ttcgaggagt ctctttgttg aggacttttg
241 agttctccct tgaggctccc acagatacaa taaatatttg agattgaacc ctgctcagta
301 tctgtgtaat cttttttacc tgtgaggtct cggaatccgg gccgagaact tcgcagttgg
361 cgcccgaaca gggacttgat tgagagtgat tgaggaagtg aagctagagc aatagaaagc
421 tgtaagcag aactcctgct gacctaataa ggaagcagt agcagacgct gctaacagtg
481 agtatctcta gtgaagcgga ctcgagctca taatcaagtc attgtttaaa ggcccagata
541 aattacatct ggtgactctt cgcggaacct caagccagga gattcgccga gggacagtca
601 acaaggtagg agagattcta cagcaacatg ggaatggagc aggggcgaga ttggaaaatg
661 gccattaaga gatgtagtaa tgttgctgta ggagtagggg ggaagagtaa aaaatttggg
721 gaaggggaatt tcagatgggc cattagaatg gctaattgat ctacaggacg agaacctggt
781 gatataccag agactttaga tcaactaagg ttggttattt gcgatttaca gaaagaaga
841 gaaaaatttg gatctagcaa agaaattgat atggcaattg tgacattaaa agtctttgcg
901 gtagcaggac ttttaaatat gacggtgtct actgctgctg cagctgaaaa tatgtattct
961 caaatgggat tagacactag gccatctatg aaagaagcag gtggaaaaa ggaaggccct
1021 ccacaggcat atcctattca aacagtaaat ggagtaccac aatatgtagc acttgaccca
1081 aaaatggtgt ccatttttat ggaaaaggca agagaaggac taggaggtga ggaagttaa
1141 ctatggttta ctgccttctc tgcaaattta acacctactg acatggccac attaataatg
1201 gccgcaccag ggtgctgctg agataaagaa atattggatg aaagcttaaa gcaactgaca
1261 gcagaatatg atcgcacaca tcccctgat gctcccagac cattacccta tttactgca
1321 gcagaaatta tgggtatagg attaaactcaa gaacaacaag cagaagcaag atttgacca
1381 gctaggatgc agtgtagagc atggtatctc gaggcattag gaaaattggc tgccataaaa
1441 gctaagtctc ctcgagctgt gcagttaaga caaggagcta aggaagatta ttcattcttt
1501 atagacagat tgtttgcccc aatagatcaa gaacaaaata cagctgaagt taagtatat
1561 ttaaaacagt cattgagcat agctaattgct aatgcagact gtaaaaaggc aatgagccac
1621 cttaagccag aaagtaacct agaagaaaag ttgagagctt gtcaagaaat aggtcacca
1681 ggatataaaa tgcaactctt ggcagaagct cttacaaaag ttcaagtagt gcaatcaaaa
1741 ggatcaggac cagtgtgttt taattgtaaa aaaccaggac atctagcaag acaatgtaga
1801 gaagtgaaaa aatgtaataa atgtgaaaaa cctggtcatt tagctgccaa atgttgcaa
1861 ggaaatagaa agaattcggg aaactggaag gcggggcgag ctgcagcccc agtgaatcaa
1921 atgcagcaag cagtaatgcc atctgcacct ccaatggagg agaaactatt ggatttataa
1981 attataataa agtaggtact actacaacat tagaaaagag gccagaaata ctcatatattg
2041 taaatggata tcctataaaa tttttattag acacaggagc agatataaca atttttaata
2101 ggagagattt tcaagtaaaa aattctatag aaaatggaag gcaaaatatg attggagtag
2161 gaggaggaaa gagaggaaca aattatatta atgtacattt agagattaga gatgaaaatt
2221 ataagacaca atgtatatat ggtaattgtt gtgtcttaga agataactca ttaatacaac
2281 cattattagg gagagataat atgattaaat tcaatattag gttagtaatg gctcaaattt
2341 ctgataagat tccagtagta aaagtaaaaa tgaaggatcc taataaagga cctcaataaa
2401 aacaatggcc attaacaatg gaaaaaattg aagccttaac agaaatagta gaaagactag
2461 aaagagaagg gaaagtaaaa agagcagatc caaataatcc atggaataca ccagtatttg
2521 ctataaaaaa gaaaagtgga aaatggagaa tgctcataga ttttagagaa ttaaacaaac
2581 taactgagaa aggagcagag gtccagttgg gactacctca tcctgctggt ttacaaataa
2641 aaaaacaagt aacagtatta gatatagggg atgcatattt caccattcct cttgatccag
2701 attatgctcc ttatacagca tttactttac ctagaaaaaa taatgcggga ccaggaagga
2761 gatttggtgt gtgtagtcta ccacaaggct ggatttttaag tccattgata tatcaaagta
2821 cattagataa tataatacaa ccttttatta gacaaaatcc tcaatagat atttaccat
2881 atatggatga catttatata ggatcaaatt taagtaaaaa ggagcataaa gaaaaggtag
2941 aagaattaaag aaaattacta ttatggttgg gatttgaaac tccagaagat aaattacagg
3001 aagaaccccc atatacatgg atgggttatg aattacatcc attaacatgg acaatacaac
3061 agaaacagtt agacattcca gaacagccca ctctaaatga gttgcaaaaa ttagcaggaa
3121 aaattaattg ggctagccaa gctattccag acttgagtat aaaagcatta actaacatga
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FIG 1

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3181 tgagaggaaa tcaaacctta aattcaacaa gacaatggac taaagaagct cgactggaag
3241 tacaaaaggc aaaaaaggct atagaagaac aagtacaact aggatactat gaccccagta
3301 aggagttata tgctaaatta agtttggtgg gaccacatca aataagttat caagtatatc
3361 agaaggatcc agaaaagata ctatggtatg gaaaaatgag tagacaaaag aaaaaggcag
3421 aaaatacatg tgatatagcc ttaagagcat gctataagat aagagaagag tctattataa
3481 gaataggaaa agaaccaaga tatgaaatac ctacttctag agaagcctgg gaatcaaatt
3541 taattaattc accatatctt aaggccccac ctctgagggt agaatatatc catgctgctt
3601 tgaatataaa gagagcgtaa agtatgataa aagatgctcc aataccagga gcagaaacat
3661 ggtatataga tggaggtaga aagctaggaa aagcagcaaa agcagcctat tgacagata
3721 caggaaagtg gcaagtgatg gaattagaag gcagtaatca gaaggcagaa atacaagcat
3781 tattattggc attaaaagca ggatcagagg agatgaatat tataacagat tcacaatatg
3841 ttataaatat tattcttcaa caaccagata tgatggaggg aatctggcaa gaagttttag
3901 aagaattgga gaagaaaaca gcaatattta tagattgggt cccaggacat aaaggatttc
3961 caggaaatga ggaagtagat aagctttgtc aaacaatgat gataatagaa ggggatggga
4021 tattagataa aaggtcagaa gatgcaggat atgatttatt agctgcaaaa gaaatacatt
4081 tattgccagg agaggtaaaa gtaataccaa caggggtaaa gctaattgtg cctaaaggat
4141 attggggatt aataatagga aaaagctcga tagggagtaa aggattggat gtattaggag
4201 gggtaataga cgaaggatat cgaggtgaaa ttggagtaat aatgattaat gtatcaagaa
4261 aatcaatcac cttaatggaa cgacaaaaga tagcacaatt aataatattg ccttgtaaac
4321 atgaagtatt agaacaagga aaagtagtaa tggattcaga gagaggagac aatggttatg
4381 ggtcaacagg agtattctcc tcttgggttg acagaattga ggaagcagaa ataatcatg
4441 aaaaatttca ctcagatcca cagtacttaa ggactgaatt taatttacct aaaatggtag
4501 cagaagagat aagacgaaaa tgcccagtat gcagaatcag aggagaacaa gtgggaggac
4561 aattgaaaat agggcctggt atctggcaaa tggattgcac acactttgat ggcaaaaata
4621 ttcttggtgg tatacatgtg gaatcaggat atatatgggc acaaataatt tctcaagaaa
4681 ctcttgactg tacagttaaa gctgtcttac aattgttgag tgctcataat gttactgaat
4741 tacaaacaga taatggacca aattttaaaa atcaaaagat ggaaggagta ctcaattaca
4801 tgggtgtgaa acataagttt ggtatcccag ggaaccaca gtcacaagca ttagtgtgaa
4861 atgtaaatca tacattaaaa gtttggattc ggaattttt gcctgaaaca acctccttgg
4921 ataatgcctt atctctcgct gtacatagtc tcaattttta aagaagaggt aggataggag
4981 ggatggcccc ttatgaatta ttagcacaac aagaatcctt aagaatacaa gattattttt
5041 ctgcaatacc acaaaaattg caagcacagt ggatttatta taaagatcaa aaagataaga
5101 aatggaaagg accaatgaga gtagaatact ggggacaggg atcagtatta ttaaaggatg
5161 aagagaaggg atattttctt atacctagga gacacataag gagagttcca gaacctgcg
5221 ctcttcctga aggggatgag tgaagaagat tggcaggtaa gtagaagact ctttgacgtg
5281 ctccaaggag gagtaaatag cgctatgcta tacatatcta ggctacctcc ggatgaaaga
5341 gaaaagtata aaaaagactt caagaaaaga ctttttgaca cagaaacagg atttataaag
5401 agactacgga aagctgaagg aataaaatgg agctttcata ctagagatta ttacatagga
5461 tatgtcagag aaatgggtgc aggatccact acatcattaa gtctaaggat gtatatatat
5521 ataagtaacc cactatggca ttctcagtat cgtccagggt tgaaaaattt caataaggaa
5581 tggccttttg taaatatgtg gataaaaaaca ggatttatgt gggatgatat tgaaaaacaa
5641 aatatttgta taggaggaga agtttcacca ggtatgggac cagggatggt aggtatagca
5701 ataaaagctt ttagttgtgg cgaaagaaag attgaggcta ctctgtaat gattataaga
5761 ggagaaatag atccaaaaaa atggtgcgga gattgttgga atttaattgt tcttagaaac
5821 tcacctocaa agactttaca aagactcgct atgttggcgt gtggcgtgcc ggctaagaag
5881 tggcagggat gctgtaatca acgctttgtt tctccttaca gaacgcctgc tgatttagag
5941 gtcatccaat ccaagcccag ctggaacctg ttatggtcgg gagaattatg aatggaagac
6001 ataatagtat tattcaatag ggtcactgag aaactagaaa aagaattagc tatcagaata
6061 tttgtattag cacatcaatt agaaagggac aaagctatta gattactaca aggattatft
6121 tggagatata gatttaagaa accccagata gattattgtt tatgttggtg gtgttgcaaa
6181 ttctattatt ggcagttgca atctacatta tcaataacta ctgcttagaa atatttagat
6241 taatatttca tttgcaacaa taagaatggc agaaggattt gcagccaata gacaatggat
6301 aggactagaa gaagctgaag agttattaga ttttgatata gcaacacaaa tgagtgaaga

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FIG 1
continued

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6361 aggaccacta aatccaggag taaacccatt tagggtagct ggaataacag aaaaagaaaa
6421 gcaaaactac tgtaacatat tacaacctaa gttacaagat ctaaggaacg aaattcaaga
6481 ggtaaaactg gaagaaggaa atgcaggtaa gtttagaaga gcaagatttt taagggtattc
6541 tgatgaaagt gtattgtccc tgggtcatgc gttcatagga tattgtatat atttaggtaa
6601 tcgaaataag ttaggatctt taagacatga cattgatata gaagcaccoc aagaagagtg
6661 ttataataat agagagaagg gtacaactga caatataaaa tatggtagac gatgttgctt
6721 aggaacggtg actttgtacc tgattttatt tataggaata ataatatatt cacagacaac
6781 caacgctcag gtagtatgga gacttccacc attagtagtc ccagtagaag aatcagaaat
6841 aattttttgg gattgttggg caccagaaga acccgctgt caggactttc ttggggcaat
6901 gatacatcta aaagctaaga caaatataag tatacgagag ggacctacct tggggaattg
6961 ggctagagaa atatgggcaa cattattcaa aaaggctact agacaatgta gaagaggcag
7021 aatatggaaa agatggaatg agactataac aggaccatca ggatgtgcta ataacacatg
7081 ttataatgtt tcagtaatag tacctgatta tcagtgttat ttagatagag tagatacttg
7141 gttacaaggg aaaataaata tatcattatg tctaacagga ggaaaaatgt tgtacaataa
7201 agttacaaaa caattaagct attgtacaga ccattacaa atcccactga tcaattatac
7261 atttggacct aatcaaacat gtatgtggaa tacttcacaa attcaggacc ctgaaatacc
7321 aaaatgtgga tgggtggaatc aaatggccta ttataacagt tgtaaattgg aagaggcaaa
7381 agtaaagttt cattgtcaaa gaacacagag tcagcctgga tcatggttta gagcaatctc
7441 gtcattgaaa caaagaaata gatgggagtg gagaccagat ttgaaagta aaaagggtgaa
7501 aatatctcta cagtgcata gcacaaaaaa cctaaccttt gcaatgagaa gttcaggaga
7561 ttatggagaa gtaacgggag cttggataga gtttggatgt catagaaata aatcaaaact
7621 tcatgctgaa gcaaggttta gaattagatg tagatggaat gtagggagta atacctcgct
7681 cattgataca tgtggaaaca ctcaaaaagt ttcagggtgcg aatcctgtag attgtaccat
7741 gtattcaaat aaaatgtaca attgttcttt acaaaaacggg tttactatga aggtagatga
7801 ccttattatg catttcaata tgaaaaaggc tgtagaaatg tataatattg ctggaaattg
7861 gtcttgtaga tctgacttgc catcgctcatg ggggtatatg aattgtaatt gtacaaatag
7921 tagtagtagt tatagtggta ctaaaatggc atgtcctagc aatcgaggga tcttaaggaa
7981 ttggtataac ccagtggcag gattacgaca atccttagaa cagtatcaag ttgtaaaaca
8041 accagattac ttagtggctc cagaggaagt catggaatat aaacctagaa ggaaaagggc
8101 agctattcat gttatgttgg ctcttgtagc agtattatct attgccggtg cagggacggg
8161 ggctactgct atagggatgg taacacaata ccaccaagtt ctggcaacc atcaagaagc
8221 tgtagaaaag gtgactgaag ccttaaagat aaacaactta agattagtta cattagagca
8281 tcaagtacta gtaataggat taaaagtaga agctatggaa aaatttttgt atacagcttt
8341 cgctatgcaa gaattaggat gtaatcaaaa tcaatttttc tgcaaaatcc ctctgagtt
8401 gtggacaagg tataatatga ctataaatca aacaatatgg aatcatggaa atataacttt
8461 gggggaatgg tataaccaaa caaaagattt acaacaaaag ttttatgaaa taataatgga
8521 catagaacaa aataatgtac aagggaagaa agggatacaa caattacaaa agtgggaaga
8581 ttgggtagga tggataggaa atattccaca atattttaaag ggactattgg gaggtatctt
8641 gggaatagga ttaggagtgt tattattgat tttatgttta cctacattgg ttgattgtat
8701 aagaaattgt atccacaaga tactaggata cacagtaatt gcaatgcctg aagtagaagg
8761 agaagaaata caaccacaaa tggaattgag gagaaatggt aggcaatgtg gcatgtctga
8821 aaaagaggag gaatgatgaa gtatctcaga cttattttat aaggagata ctgtgctgag
8881 ttcttccctt tgagggaagg atgtcatatg aatccatttc gaatcaaat aaactaataa
8941 agtatgtatt gtaaggtaaa aggaaaagac aaagaagaag aagaaagaag aagccttca
9001 agaggatgat gacagagtta gaagatcgct tcaggaagct atttggcacg acttctacaa
9061 cgggagacag cacagtagat tctgaagatg aacctcctaa aaaagaaaaa aggggtggact
9121 gggatgagta ttggaaccct gaagaaatag aaagaatgct tatggactag ggactgttta
9181 cgaacaaatg ataaaaggaa atagctgagc atgactcata gttaaagcgc tagcagctgc
9241 ctaaccgcaa aaccacatcc tatggaaagc ttgctaataa cgtataagtt gttccattgt
9301 aagagtatat aaccagtgtt ttgtgaaact tcgaggagtc tctttgttga ggacttttga
9361 gttctccctt gaggctccca cagatacaat aaatatitga gattgaaccc tgtcgagtat
9421 ctgtgtaatc ttttttacct gtgaggtctc ggaatccggg ccgagaactt cgca

```

FIG 1
continued

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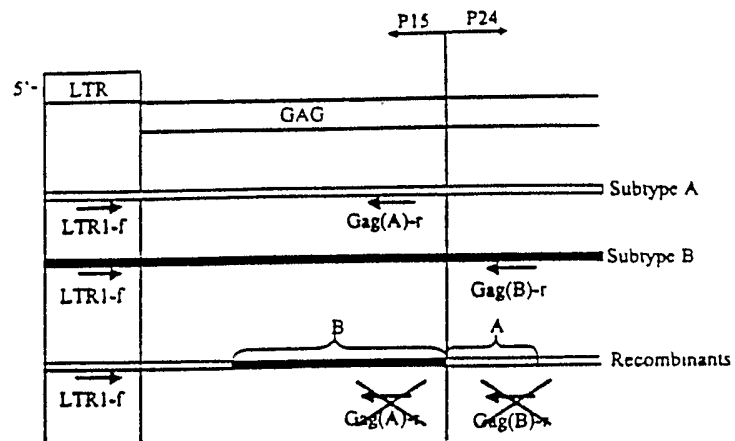


FIG 2

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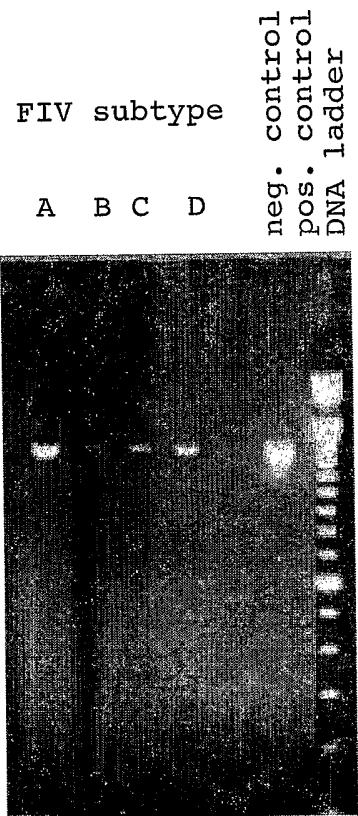


FIG 3

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FIV subtypes:

A B B C D B B B B

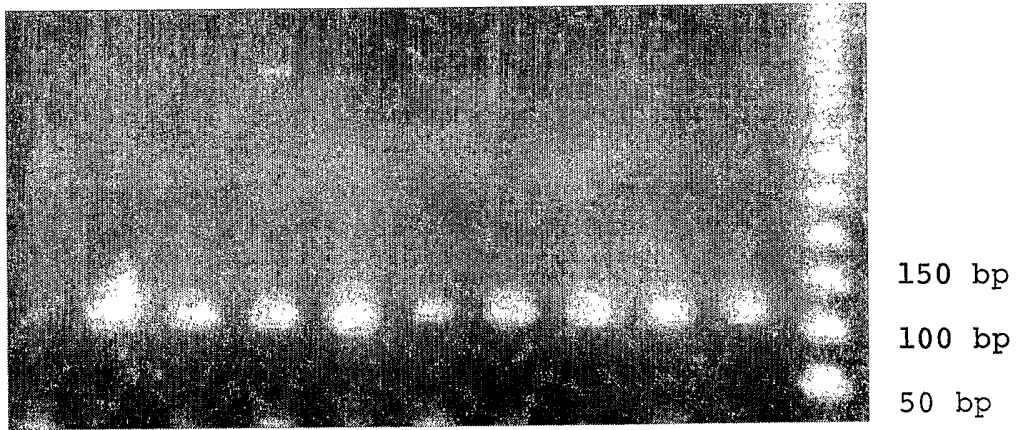


FIG 4

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Section 1									
	1	10	20	30	40	50	60	75	
Petaluma-first2kb	(1)	TGGGATGAGTATTGGAA	CCCTGAAAGAAATAGAAAGAAATGCTTAT	TGGACTAGGGA	CTGTTTACGAA	CAAAATGATAA			
LTR1-f	(1)	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Consensus	(1)	-----	-----	-----	-----	-----	-----	-----	
Section 2									
	76	90	100	110	120	130	140	150	
Petaluma-first2kb	(76)	AAGGAAATAGCTGAGCATGACTCATAGTTAAAGCGTAGCAGCTGCC	TAAACCGCAAAA	CCACATCC	TATGGAAA	G			
LTR1-f	(1)	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Consensus	(76)	-----	-----	-----	-----	-----	-----	-----	
Section 3									
	151	160	170	180	190	200	210	225	
Petaluma-first2kb	(151)	CTTGCTAAATGACGTATAAGTTGTTCCCATTTGTAAGAGTATATAA	CCAGTGTCTTTGTGAAA	CTTCGAGGAGTCTCTT					
LTR1-f	(21)	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Consensus	(151)	-----	-----	-----	-----	-----	-----	-----	
Section 4									
	226	240	250	260	270	280	290	300	
Petaluma-first2kb	(226)	TGTTGAGGAC	TTTTTGAGTTCTCCCTTGAGGCTCCCA	CAGATACAAATAAA	TATTTGAGATTGA	ACCCCTGT	CGAGTA		
LTR1-f	(21)	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(1)	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(1)	-----	-----	-----	-----	-----	-----	-----	
Consensus	(226)	-----	-----	-----	-----	-----	-----	-----	

FIG 5

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		Section 5													
		301	310	320	330	340	350	360	375						
Petaluma-first2kb	(301)	TCTGTGTAATCTTTT	TTCCTGTGAGGTCTCGGAATCCGGGCCGAGAACTTCGCAGTTGGCCCGAACAGGGAC												
LTR1-f	(21)														
LTR2-f	(17)	TCTGTGTAA													
Gag1-r (complementary)	(1)														
Gag635f	(1)														
Gag718r (complementary)	(1)														
Consensus	(301)														
		Section 6													
		376	390	400	410	420	430	440	450						
Petaluma-first2kb	(376)	TTGATTGAGAGTGAAGTGAAGCTAGAGCAATAGAAAGCTGTTAAGCAGAACTCCTGCTGACCTAAATA													
LTR1-f	(21)														
LTR2-f	(26)														
Gag1-r (complementary)	(1)														
Gag635f	(1)														
Gag718r (complementary)	(1)														
Consensus	(376)														
		Section 7													
		451	460	470	480	490	500	510	525						
Petaluma-first2kb	(451)	GGGAAGCAGTAGCAGACGCTGCTAAACAGTGAGTATCTCTAGTGAAGCGGACTCGAGCTCATAATCAAGTCATTGT													
LTR1-f	(21)														
LTR2-f	(26)														
Gag1-r (complementary)	(1)														
Gag635f	(1)														
Gag718r (complementary)	(1)														
Consensus	(451)														
		Section 8													
		526	540	550	560	570	580	590	600						
Petaluma-first2kb	(526)	TTAAAGGCCAGATAAAATTACATCTGGTGACTCTTCGCGGACCTTCAAGCCAGGAGATTCCGCCGAGGGACAGTCA													
LTR1-f	(21)														
LTR2-f	(26)														
Gag1-r (complementary)	(1)														
Gag635f	(1)														
Gag718r (complementary)	(1)														
Consensus	(526)														

FIG 5 continued

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		Section 9											
		601	610	620	630	640	650	660	675				
Petaluma-first2kb	(601)	ACAAGGTAGGAGAGATTCTACAGCAACATGGGGAAATGGACAGGGGCGAGATTGGAAAATGGCCATTAAAGAGATGT											
	LTR1-f	(21)											
	LTR2-f	(26)											
	Gag1-r (complementary)	(1)											
	Gag635f	(1)											
Gag718r (complementary)	(1)	-AACATGGGGAATGGACAGG-											
Consensus	(601)												
		Section 10											
		676	690	700	710	720	730	740	750				
Petaluma-first2kb	(676)	AGTAATGTTGCTGTAGGAGTAGGGGGAAGAGTAAAAAATTTGGAGAAGGGAATTTTCAGATGGGCCATTAGAATG											
	LTR1-f	(21)											
	LTR2-f	(26)											
	Gag1-r (complementary)	(1)											
	Gag635f	(20)											
Gag718r (complementary)	(1)												
Consensus	(676)												
		Section 11											
		751	760	770	780	790	800	810	825				
Petaluma-first2kb	(751)	GCTAATGTATCTACAGGACGAGAACCTGGTGATATATCCAGAGACTTTAGATCAACTAAGGTTGGTTATTTGCCGAT											
	LTR1-f	(21)											
	LTR2-f	(26)											
	Gag1-r (complementary)	(1)											
	Gag635f	(20)											
Gag718r (complementary)	(1)	-ACGAGAACCTGGTGATATACC-											
Consensus	(751)												
		Section 12											
		826	840	850	860	870	880	890	900				
Petaluma-first2kb	(826)	TTACAAGAAAGAGAGAAAAATTTGGATCTAGCAAGAAAATTTGATATGGCAATTGTGACATTAAAGTCTTTGCG											
	LTR1-f	(21)											
	LTR2-f	(26)											
	Gag1-r (complementary)	(1)											
	Gag635f	(20)											
Gag718r (complementary)	(22)												
Consensus	(826)												

FIG 5 continued

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Section 17									
	1201	1210	1220	1230	1240	1250	1260	1275	
Petaluma-first2kb (1201)	GCCGCA	CCAGGT	GCCTGC	AGATAA	AGAAATA	TTGGAT	GAAAGCT	TAAAGCAA	CTGACAGCAGAGATAATGATCGC
LTR1-f (21)	-----	-----	-----	-----	-----	-----	-----	-----	-----
LTR2-f (26)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag1-r (complementary) (1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag635f (20)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag718r (complementary) (22)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Consensus (1201)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Section 18									
	1276	1290	1300	1310	1320	1330	1340	1350	
Petaluma-first2kb (1276)	ACACAT	CCCCCT	GATGCT	CCAGACCA	TATACCC	TATTTT	ACTGTCAGCAGAGAA	TTATGGGTATAGGATTA	AACTCAA
LTR1-f (21)	-----	-----	-----	-----	-----	-----	-----	-----	-----
LTR2-f (26)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag1-r (complementary) (1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag635f (20)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag718r (complementary) (22)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Consensus (1276)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Section 19									
	1351	1360	1370	1380	1390	1400	1410	1425	
Petaluma-first2kb (1351)	GAA	CAACA	AGCAGAA	GCAAGATT	TGCA	CCAGCTAGGAT	GCAGTGTAGAGCAT	GGTATCT	CGAGGCATTAGGAAAA
LTR1-f (21)	-----	-----	-----	-----	-----	-----	-----	-----	-----
LTR2-f (26)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag1-r (complementary) (1)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag635f (20)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag718r (complementary) (22)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Consensus (1351)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Section 20									
	1426	1440	1450	1460	1470	1480	1490	1500	
Petaluma-first2kb (1426)	TTGGCT	GCCATA	AAAAAGCT	AAAGTCT	CCTCGAGCT	GTGTCAGT	TAAAGACAA	AGGAGCTA	AGGAGATTATTCATCCTTT
LTR1-f (21)	-----	-----	-----	-----	-----	-----	-----	-----	-----
LTR2-f (26)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag1-r (complementary) (22)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag635f (20)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Gag718r (complementary) (22)	-----	-----	-----	-----	-----	-----	-----	-----	-----
Consensus (1426)	-----	-----	-----	-----	-----	-----	-----	-----	-----

FIG 5 continued

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Section 21										
	(1501)	1501	1510	1520	1530	1540	1550	1560	1575	
Petaluma-first2kb	(1501)	ATAGACAGATTGTTTGGCCCAATAGATCAAGAA	CAAAATACAGCTGAAGTTAAAGTTATATTTAAAA	CAGTCAATG						
LTR1-f	(21)	-----	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(26)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(20)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus (1501)		-----	-----	-----	-----	-----	-----	-----	-----	
Section 22										
	(1576)	1576	1590	1600	1610	1620	1630	1640	1650	
Petaluma-first2kb	(1576)	AGCATAGCTAATGCTAATGCAGACTGTAAAAAGGCAATGAGCCACCTTAAGCCAGAAAGTACCCCTAGAAAGAAAAG								
LTR1-f	(21)	-----	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(26)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(20)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus (1576)		-----	-----	-----	-----	-----	-----	-----	-----	
Section 23										
	(1651)	1651	1660	1670	1680	1690	1700	1710	1725	
Petaluma-first2kb	(1651)	TTTGAGAGCTTGTCAAGAAATAGGCTCAC CAGGATATAAAATGCAACTTTGGCAGAAAGCTCTTTACAAAAGTTCAA								
LTR1-f	(21)	-----	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(26)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(20)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus (1651)		-----	-----	-----	-----	-----	-----	-----	-----	
Section 24										
	(1726)	1726	1740	1750	1760	1770	1780	1790	1800	
Petaluma-first2kb	(1726)	GTAGTGCAATCAAAAGGATCAGGACCAGTGTGTTTAAATTGTAATAAACCCAGGACATCTAGCAAGACAAATGTAGA								
LTR1-f	(21)	-----	-----	-----	-----	-----	-----	-----	-----	
LTR2-f	(26)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag1-r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag635f	(20)	-----	-----	-----	-----	-----	-----	-----	-----	
Gag718r (complementary)	(22)	-----	-----	-----	-----	-----	-----	-----	-----	
Consensus (1726)		-----	-----	-----	-----	-----	-----	-----	-----	

FIG 5 continued

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		Section 25																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
		1801	1810	1820	1830	1840	1850	1860	1875																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
Petaluma-firs2kb	(1801)	GAA	GTG	AAAAAA	TG	TAA	TAA	ATGT	GAAAA	CCT	GGT	CAT	G	TAG	CTG	CC	CAA	ATG	T	TGG	CA	AG	G	A	A	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G	A	A	T	A	G	A	A	A	G

FIG 5 continued

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/000939

A. CLASSIFICATION OF SUBJECT MATTER IPC(7): C07H 21/04, C12Q 1/68 According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC(7): C07H 21/04, C12Q 1/68 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used) Delphion, Biosis, CaPlus, STN Registry, Genbank and GeneSeq (Searched Seq Id Nos 2 to 6) Keywords: FIV, gag, primer, diagnosis.				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	EP 1074625 A2 (PFIZER PRODUCTS INC. US) 7 February 2001 See page 6, paragraph [0039], page 18, section 5.4 and SEQ ID NOs 2, 3 and 48.	1 to 4, 7 to 11, 13 to 16 and 18 to 20		
X	WO 00/44935 A2 (BAVARIAN NORDIC RESEARCH INSTITUTE A/S [DK/DK]) 3 August 2000. See page 4, line 23 to page 5, line 7, page 7, line 23 to page 8, line 4 and SEQ ID NOs 1 and 6.	1 to 7, 10, 12 to 15, 17 and 19		
X	LAWSON, M., et al., "The detection and quantification of feline immunodeficiency provirus in peripheral blood mononuclear cells using the polymerase chain reaction", Veterinary Microbiology. 1993, Vol. 38 (1-2), pages 11-21. Whole Document	1, 2, 7, 10 and 13		
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.				
<table border="0"> <tr> <td> * Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> </tr> </table>			* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
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Date of the actual completion of the international search 14 September 2005 (14-09-2005)		Date of mailing of the international search report 20 October 2005 (20-10-2005)		
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476		Authorized officer Nathalie Chartrand (819) 994-2341		

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International application No.
PCT/CA2005/000939

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOHDATSU T., et al., "Detection of feline immunodeficiency proviral DNA in peripheral blood lymphocytes by the polymerase chain reaction", Veterinary Microbiology. 1992, Vol. 30 (2-3), pages 113-123. Whole Document.	1, 2, 10 and 13
X	KIDNEY, BA., et al., "Evaluation of formalin-fixed paraffin-embedded tissues obtained from vaccine site-associated sarcomas of cats for DNA of feline immunodeficiency virus", American Journal of Veterinary Research. 2000, Vol. 61, no. 9, pages 1037-1041. Abstract and Materials and Methods	1, 2, 10 and 13
Y	EP 1374910 A1(WYETH, Madison, New Jersey US) 2 January 2004. See page 3, lines 35 to 38 and SEQ ID NO 10.	3, 14 and 19

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national application No.
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