

VIRUSES AND THEIR USE AS A VACCINE AGAINST
FELINE INFECTIOUS PERITONITIS VIRUS DISEASE

5 Field of the Invention

The present invention pertains to the prophylaxis of disease caused by feline infectious peritonitis virus (FIPV), using recombinant raccoon pox viruses (RRPVs) expressing the nucleocapsid and transmembrane proteins of FIPV as vaccines.

Background of the Invention

10 Feline infectious peritonitis virus (FIPV) induces a systemic infection in cats that is often fatal. The effusive form of the disease is characterized by accumulation of fibrinous ascitic fluid. The non-effusive form of the disease is characterized by
granulomatous lesions in multiple organs including, but not limited to, liver, spleen, kidneys, lung, and intestines. Reviewed in Barlough, J.E. and C. A. Stoddart. Feline
15 Coronaviral Infections in C.E. Greene (Ed.). Infectious Diseases of the Dog and Cats. W.B. Saunders Co., Philadelphia, PA, 1990, pp. 300-312.

Feline infectious peritonitis virus is a coronavirus composed of three major structural proteins: The S (spike) protein, the E1 or M (transmembrane) protein, and the N (nucleocapsid) protein. Venema et al., Virology 181: 327-335, 1991 and Dale, et al.,
20 EPO 0,376, 744.

Prior vaccines intended to prevent FIPV infection have actually been shown to exacerbate the disease caused by this virus. Pedersen, N.C. and J.W. Black, Am. J. Vet. Res. 44: 229-234, 1983; Vennema H., et al., J. Virol. 64: 1407-1409, 1990; Barlough, J.E., Can. J. Comp. Med. 49: 303-307, 1985; Barlough J.E. et al.,
25 Lab. Anim. Sci. 34: 592-597, 1984; Stoddart, C.A., et al., Res. Vet. Sci. 45: 383-388, 1988; and Pedersen, N.C., Adv. Vet. Sci. Comp. Med. 33: 413-428, 1989. This

This Sheet Has Been Replaced



REPLACED SHEETS		SG
☒	Description : paged ...1... to paged ...2...	
☒	Claim(s) : paged ...3... to paged ...3...	
☒	Drawing(s) : paged ...4... to paged ...15/16...	
☒	Abstract : paged ...34... to paged ...34...	
DATE OF RECEIPT		

phenomenon apparently reflects an immune enhancement of infection mediated by immunoglobulins produced in response to the virus, in particular by those antibodies directed against the S protein. Olsen C. W. et al., J. Virol. 4: 175-189, 1981. Therefore, the best candidate vaccine for prophylaxis of this disease would be a preparation that induces strong cell-mediated immunity in the absence of enhancing antibodies. This could be accomplished with a vaccine that lacks the outer envelope protein but contains the other structural proteins of FIPV (N and E1). Prior attempts to vaccinate cats with a recombinant vaccinia virus expressing the N or E1 proteins of FIPV, however, have failed to induce strong protective immunity. Venema et al., Virology 181:327-335, 1991 and Dale, et al., European Patent Application 0,376,744. See also, Venema, European Patent Application 0,411,684.

What is needed in the art, therefore, is an effective vaccine against FIPV that utilizes the N and E1 proteins, or segments therefrom, as immunogens.

Summary of the Invention

The present invention pertains to the induction of protective immunity to FIPV in cats. One object of the invention is to provide recombinant raccoon poxviruses containing the genes for the FIPV N or M/E1 proteins (RRPV-N and RRPV-E1, respectively).

A further object of the invention is to provide a feline vaccine comprising RRPV-N or RRPV-E1, either singly or in combination, or in combination with other viruses, bacteria, or fungi that have been inactivated or attenuated. A still further object of the invention is to provide a method for preventing disease caused by FIPV, by administering to a feline in need of such treatment a vaccine comprising RRPV-N, RRPV-E1, or combinations thereof.

These and other objects and advantages, which will be apparent from this specification, are achieved by the invention described below.



- 3 -

Description of the Drawings

Figure 1 illustrates the nucleotide and amino acid sequence of the FIPV E1 protein and the FIPV N protein (Figures 1A and 1B, respectively.)

5 Figure 2 illustrates the plasmid used to clone the genes encoding the FIPV E1 and N proteins.

Figure 3 schematically shows the pSC11 transfer plasmids used to create RRPVs encoding the FIPV E1 and N proteins (Figs 3B and 3C, respectively.)

Figure 4 illustrates the nucleotide sequence of pSC11 FIPV E1 and pSC11 FIPV N (Figures 4A and 4B, respectively.)

10 Figure 5 is a photograph of an ethidium bromide-stained agarose gel showing the analysis of RRPV-FIPV N and RRPV-FIPV E1 by polymerase chain reaction.

Figure 6 is an immunoblot illustrating the detection of FIPV N and E1 proteins in virally infected cell lysates.

15

Detailed Description of the Invention

The vaccine of the present invention may be prepared by creating recombinant raccoon poxviruses (RRPVs) containing the genes encoding the N or E1 proteins of FIPV or immunogenic fragments thereof. These genes are first inserted into a transfer plasmid, which is then introduced into appropriate host cells that have been previously infected with a raccoon poxvirus. As a result, the DNA from the transfer plasmid is incorporated into the poxvirus DNA by homologous recombination, producing the RRPVs that are released from the cells.

20 DNA encoding the FIPV N or E1 proteins is inserted into the transfer plasmid immediately downstream of a poxvirus promoter. In a preferred embodiment, the early/late 7.5 Kd protein promoter of vaccinia virus is used; however, alternate promoter elements that are functional in poxviruses can also be used.

25 The preferred transfer plasmid also contains a beta-galactosidase marker gene, which allows for selection and detection of the plasmid DNA sequences in

This Sheet Has Been Replaced



- 4 -

recombinant viruses. It will be obvious to one skilled in the art that alternative selectable marker genes, such as the neomycin resistance gene or the E. coli gpt gene or others, can be used to practice the invention. Flanking the foreign gene of interest and the selectable marker gene are thymidine kinase DNA sequences, which facilitate recombination integration of the plasmid DNA sequences into the raccoon poxvirus DNA.

Recombinant viruses expressing the FIPV N or E1 genes are prepared by first infecting a susceptible cell line such as Vero (ATCC CCL 81), BSC-1 (ATCC CCL 26), RAT-2 (ATCC CRL 1764), or CRFK (ATCC CCL 941) with wild type raccoon poxvirus (ATCC VR-838 or similar isolates, such as, for example, RCNV71-I-85A). Transfer plasmid DNA containing the E1 or N gene is then transfected into the infected cells using cationic liposome-mediated transfection, or other suitable techniques such as electroporation or calcium phosphate precipitation. Virus replication is allowed to proceed until cytopathic effects are noted in all cells.

Incorporation of the FIPV E1 or N genes into poxvirus DNA is accompanied by disruption of the viral thymidine kinase gene. Therefore, virus harvested from this infection may be isolated by selecting for the absence of a thymidine kinase gene; this is achieved by growth on tk- RAT-2 cells (ATCC CRL 1764) in the presence of 5-bromodeoxyuridine. Viruses containing a gene insert from the transfer plasmid are further identified by the appearance of a blue plaque color when grown in the presence of a chromogenic substrate for beta-galactosidase such as X-gal.

Viral plaques that survive these selection and screening procedures are then subjected to several cycles of plaque purification. Subsequently, the presence of the E1 or N genes is confirmed by polymerase chain reaction technology, and the presence of E1 or N protein is confirmed by immunoblot analysis using specific antibodies. These viruses are designated RRPV-FIPV E1 and RRPV-FIPV N, respectively.

In a further embodiment of the present invention, the genes encoding N and E1 were inserted into a single transfer plasmid. Introduction of this plasmid into

This Sheet Has Been Replaced



- 5 -

cells previously infected with wild-type raccoon poxvirus results in the production of recombinant viruses that express both proteins simultaneously (RRPV-FIPV E1/N).

In a still further embodiment, RRPVs can be produced that express less-than-full-length segments of the FIPV E and N proteins. The techniques used to engineer transfer plasmids encoding partial sequences of E1 and N are well-known and widely used in the art, as are the methods for production and screening of RRPVs as detailed in this specification. For example, introduction of oligonucleotides containing a stop codon at various points along E1 or N DNA will produce a nested set of carboxyterminal-truncated versions of that gene, which can then be incorporated into RRPVs. It will be apparent to one of ordinary skill in the art that systematic screening of such recombinant RRPVs can establish whether the intact protein, or subfragments thereof, are most preferred in practicing the present invention. Furthermore, as stated above, DNA encoding different fragments of E1 and N can be used in a combination vaccine after incorporation into the same, or different, RRPVs.

For vaccine preparation, susceptible cells such as those listed above are infected with RRPVs at a multiplicity of infection (MOI) of 0.1 infectious units/cell or less. In this specification, an infectious unit is defined as a Tissue Culture Infectious Dose (TCID₅₀), an amount of virus yielding 50% infection under defined conditions. A method for TCID₅₀ determination is detailed in Example 1 below. When cytopathology is noted in >90% of the cells, the infected cells and extracellular fluids (both of which contain viruses) are harvested as a single virus-cell lysate.

The highly concentrated virus stock to be used as a vaccine may be stored frozen (-50°C or colder) or lyophilized until the time of use. Compounds such as NZ-amine, dextrose, gelatin or others designed to stabilize the virus during freezing and lyophilization may be added. The virus initially present in the virus-cell lysate may be further concentrated using commercially available equipment.

Typically, the concentration of virus in the vaccine formulation will be a minimum of 10^{6.5} TCID₅₀ per dose, but will typically be in the range of 10^{7.0} to 10^{9.0} TCID₅₀ per dose. At the time of vaccination, the virus is thawed (if frozen) or, if

This Sheet Has Been Replaced



- 6 -

lyophilized, is reconstituted with a physiologically-acceptable carrier such as deionized water, saline, phosphate buffered saline, or the like.

The present invention is not limited to native (i.e. replication-competent) RRPVs. The virus-cell lysate can be subjected to treatments commonly used in the art to inactivate viruses. A composition comprising inactivated virus and expressed protein will be effective in eliciting protective immunity against FIPV if it contains a sufficient quantity of FIPV protein. This type of vaccine would provide added assurance that recipient felines will not be exposed to infectious FIPV as a consequence of vaccination. In addition, a physiologically-acceptable adjuvant may be added to the virus, such as EMA 31 (Monsanto Co., St. Louis, MO), NEOCRYL (Polyvinyl Chemical Industries, Wilmington, MA), MVP (Modern Veterinary Products, Omaha, NE), Squalene, Pluronic L121, or the like.

Individual raccoon poxviruses expressing the N or EI genes may be mixed together for vaccination. Furthermore, the virus may be mixed with additional inactivated or attenuated viruses, bacteria, or fungi such as feline leukemia virus, feline panleukopenia virus, feline rhinotracheitis virus, feline calicivirus, feline immunodeficiency virus, feline herpesvirus, feline enteric coronavirus, feline *Chlamydia psittaci*, *Microsporium canis*, or others. In addition, antigens from the above-cited organisms may be incorporated into combination vaccines. These antigens may be purified from natural sources or from recombinant expression systems, or may comprise individual subunits of the antigen or synthetic peptides derived therefrom.

In a further embodiment of the present invention, live or inactivated RRPV virus-cell lysates can be incorporated into liposomes, or encapsulated in peptide-, protein-, or polysaccharide-based microcapsules prior to administration, using means that are known in the art.

The final vaccine is administered to cats in a volume that may range from about 0.5 to about 5 ml. The vaccine can be administered by subcutaneous, intramuscular, oral intradermal, or intranasal routes. The number of injections and their temporal spacing may be varied. One to three vaccinations administered at intervals of

This Sheet Has Been Replaced



- 7 -

one to three weeks are usually effective.

The following examples are intended to further illustrate the invention without limiting its scope. The techniques used to infect and transfect cells, plaque purify virus, perform immunoblot analysis are widely practiced in the art.

5

Example 1

GENERATION OF RECOMBINANT RACCOON POX VIRUSES EXPRESSING FIPV N AND E1 GENES

1. Cloning of FIPV N and E1 Genes and Preparation of Transfer Plasmids

10

The sequences of the E1 and N genes used in the present invention are shown in Figures 1A and 1B, respectively, of the specification. The methods for cloning of the N and E1 genes of FIPV and their insertion into a pSC11 transfer vector are detailed in European Patent Application 0,376,744, which is incorporated by reference. The plasmid used to clone the cDNA for the E1 and N genes is shown in Figure 2. The pSC11 plasmids carrying the E1 and N genes are shown in Figures 3B and 3C, respectively. The sequences of these plasmids are shown in Figures 4A and 4B.

15

20

To construct a pSC11 transfer plasmid containing both N and E1 genes, a 1.0 kb DNA fragment containing the vaccinia 7.5 promoter and the E1 gene was inserted downstream of the N gene in pSC11-FIPV N. The resulting plasmid was designated pSC11-FIPV N/E1.

2. Preparation of Recombinant Raccoon Poxviruses (RRPVs)

Monolayers of Vero cells (ATCC CCL 81) that were 80% confluent

This Sheet Has Been Replaced



- 8 -

(approximately 5×10^6 cells/100 mm tissue culture dish) were infected for 30-60 minutes at 37°C with wild-type raccoon pox virus (ATCC VR-838) at a multiplicity of infection (MOI) of 0.1 TCID₅₀/cell. The medium (2 ml) consisted of Eagle's Minimum Essential Medium ("MEM", Gibco BRL #410-1500) containing 0.05% lactalbumin hydrolysate and 15 µg/ml gentamicin sulfate and adjusted to pH 7.2 with sodium bicarbonate. After infection, the medium was removed and the cells were transfected with the pSC11-FIPV N, pSC11-FIPV E1, or pSC11 N/E1 transfer plasmid by cationic liposome-mediated transfection using Transfectam@ (Promega Corporation, Madison, WI) and DOTAP (Boehringer Mannheim, Indianapolis, IN), respectively, per manufacturer's instructions. The cells were incubated with the DNA-liposomes mixture in 3 ml of MEM containing 5% fetal bovine serum (FBS) overnight at 37°C (5% CO₂), after which the medium was replaced with 8 ml of fresh MEM-5% FBS. The transfected cells were incubated at 37°C (5% CO₂) until greater than 80% showed cytopathic effects (CPE), which took approximately 3-4 days. The virus-cell lysates were then removed from the plates and subjected to two cycles of freeze-thawing before storage at -70°C.

3. Isolation of Recombinant Raccoon Pox Virus Carrying the FIPV N Gene

RRPVs carrying the FIPV N gene (RRPV-FIPV N) were isolated and purified from the pSC11-FIPV N- Vero cell transfection by standard viral plaque purification methods. Monolayers of Vero cells (50-80% confluent) were infected with 2 ml of ten-fold serial dilutions (10^{-1} to 10^{-3}) of the viral-cell lysates for 1 hour at 37°C. After incubation, the media was removed and the infected cells were overlaid with 8-10 ml of 1.25% Noble agar containing MEM/5% FBS. The infected cells were then incubated for 3-4 days at 37°C (5% CO₂), and overlaid again with 4 ml of 1.25% Nobel agar containing 0.5X PBS and 600 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal, States Biochemical Cleveland, Ohio). The plates were incubated at 37°C (5% CO₂) for 4-16 hours, until blue (i.e. β-galactosidase positive) viral plaques were observed. The recombinant viral plaques were picked with sterile



blunt needles attached to a 1 cc syringe, suspended in 0.5 ml of 0.25 μ g/ml trypsin, vortexed vigorously, and incubated at 37°C for 15-30 min. The disrupted viral plaques were then inoculated onto 5×10^5 Vero cells in 25 cm² flasks and incubated at 37°C (5% CO₂) until greater than 80% CPE was observed. The viral-cell lysates containing
5 RRPV-FIPV N were subjected to two cycles of freeze-thawing and stored at -70°C. Six individual RRPV-FIPV N clones were selected and plaque-purified five times as described above.

4. Isolation of Recombinant Raccoon Pox Virus Containing the FIPV E1 Gene

10 RRPVs carrying the FIPV E1 gene (RRPV-FIPV E1) were isolated and purified from pSC11-FIPV E1-transfected Vero cells using the methods described for rRPV-FIPV N, with some modifications. In this case, thymidine kinase deficient (tk-) RRPVs from the initial virus-cell lysates were selected on tk- RAT-2 cells (ATCC CRL 1764). This was performed by inoculating 1 ml of the initial virus-cell lysate onto
15 a monolayer of RAT-2 cells in a 75 cm² flask (approximately 5×10^6 cells) in the presence of 5-bromodeoxyuridine (BrdU) at 30 μ g/ml in MEM. The infected monolayer was incubated at 37°C (5% CO₂) for 3-4 days until greater than 70% CPE was observed. The tk- virus-cell lysates were subjected to two cycles of freeze-thawing before storage at -70°C. Two individual RRPV-FIPV E1 clones were selected and
20 subjected to six cycles of plaque purification as described above for RRPV-FIPV N.

5. Confirmation of FIPV N and E1 Genes in RRPV by Polymerase Chain Reaction

25 The presence of the FIPV N and E1 genes in the RRPVs was confirmed using the polymerase chain reaction (PCR). 90 μ l of a virus-cell lysate were incubated with 10 μ l of tenfold concentrated PCR lysis buffer (100 mM Tris-HCL buffer, pH 8.5; 500 mM KCl; 25 mM MgCl₂; 5% Tween 20; 3 mg/ml Proteinase K) for 16 hours at 50°C, then boiled for 10 min. 10 μ l of this lysate was used in the PCR. PCR was



- 10 -

performed in 100 μ l of 10 mM Tris-HCL buffer, pH 8.3; 50mM KCl; 200 μ M of each deoxyribonucleotide triphosphate, 1.5 mM $MgCl_2$; 30 pmoles of each oligonucleotide primer; and 2.5 units of AmpliTaq[®] DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). The primers used in the PCR for FIPV N were:

5 (1) 5'-CTCGTGGTCGGAAGAATAATGATA-3'

(2) 5'-AGCACCATAGAAAGTTGTCACATC-3',

corresponding to nucleotides 68-91 and 721-744 of the FIPV N open reading frame (primers 1 and 2, respectively). The primers used in the PCR for FIPV El were:

(3) 5'-TATGTAATGTTTCGGCTTTAGTG-3'

10 (4) 5'-GTGCTTCTGTTGAGTAATCACC-3'

corresponding to nucleotides 334-355 and 721-742 of the FIPV El open reading frame (primers 3 and 4, respectively). The PCR amplifications were performed in a DNA Thermal Cycler (Perkin-Elmer Cetus) by first heating the reaction mixes to 94°C for denaturation, and then performing 35 cycles of amplification, each consisting of 1 min at 95°C, 1 min at 55°C, 2 min at 72°C, and, on the last cycle, a final incubation of 8 min at 72°C. 10 μ l of the PCR products were resolved by electrophoresis in a horizontal-submarine 4% NuSieve agarose gel (FMC BioProducts, Rockland, ME) in TAE buffer (40 mM Tris base, 20 mM sodium acetate, 1 mM EDTA, pH 7.2) by applying 5 V/cm for 1-2 hours. The DNA products were visualized by staining the gels with ethidium bromide.

20 PCR amplifications with the FIPV N and El primers gave DNA fragments of 676 and 408 nucleotides, respectively (Figure 5). PCR amplifications using the pSC11 FIPV N and El transfer plasmids served as positive controls, and showed products of the predicted sizes. PCR amplifications using wild-type raccoon pox virus-
25 Vero cell lysates served as a negative control, and no PCR products were observed in those samples.

6. Confirmation of RRPV FIPV N and El Protein
Expression by Immunoblot Analysis

This Sheet Has Been Replaced



- 11 -

Confluent monolayers of Vero cells in a 25 cm² flask (1-2 x 10⁶ cells) were infected with clones of either RRPV-FIPV N or RRPV-FIPV E1 at an MOI of 0.1. The infected cells were incubated at 37°C (5% CO₂) for 2-3 days until approximately 80% of the cells showed cytopathic effects. A virus-cell lysate was prepared, and 20 µl of the sample were added to 5 µl of 5X Laemmli sample buffer (0.3 M Tris-HCl buffer, pH 6.8, containing 5% SDS, 50% glycerol, 0.4% bromophenol blue, and 3% 2-β-mercaptoethanol) and heated at 95°C for 5 min. The denatured protein samples were separated by SDS/polyacrylamide electrophoresis using a 4-15% gradient polyacrylamide gel as described previously. Maniatis et al., Molecular Cloning: A Laboratory Manual, 1982, Cold Spring Harbor Press. After electrophoresis, the proteins were transferred to nitrocellulose (Bio-Rad Laboratories, Hercules, CA) by electrotransfer using a Bio-Rad transfer apparatus per manufacturer's instructions. The transfer was performed in 25 mM Tris-HCl buffer, containing 0.2 M glycine and 20% methanol, for 45 minutes at 50V with constant current.

FIPV N and E1 proteins were visualized on the nitrocellulose filter using specific antibodies. Davis et al., Basic Methods in Molecular Biology, 1986, Elsevier Science Publishing Company, New York, NY. The filter was rinsed in phosphate buffered saline pH 7.4 containing 0.1% Tween-20 ("PBS-TW"), after which non-specific sites were blocked by overnight incubation at 4°C in PBS containing 1% bovine serum albumin (PBS-BSA) followed by a 15 min wash in PBS-TW. The filter was then incubated for 30 min at room temperature with anti-FIPV antibodies, which consisted of ascites fluid from a FIPV (strain 79-1146)-infected cat, diluted 1:100 in PBS-TW containing 1% BSA ("PBS-TW-BSA"). After four 5 min washes in PBS-TW, the filter was incubated for 30 min at room temperature with a secondary antibody consisting of biotin-labeled mouse anti-cat IgG antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) that had been diluted 1:2000 in PBS-TW-BSA, followed by four 5 min washes in PBS-TW. The filter was then incubated for 30 min at room temperature with horseradish peroxidase-conjugated streptavidin (Kirkegaard & Perry Laboratories Inc.) that had been diluted 1:1000 in PBS-TW. After the filter was washed four times

This Sheet Has Been Replaced



- 12 -

(5 min each) in PBS-TW, the antigen-antibody complexes were visualized with peroxidase chromogenic substrate (Kirkegaard & Perry Laboratories Inc.). Sucrose-gradient purified FIPV and wild-type raccoon pox virus-Vero cell lysates were used as the positive and negative controls, respectively. A typical immunoblot is shown in Figure 6.

7. Raccoon Poxvirus Titration

Serial tenfold dilutions of virus are prepared in MEM and inoculated in replicates of five onto Vero cells (1×10^4 cells per well) in a 96 well plate. Virus preparations may be pretreated by dilution into an equal volume of 0.5% trypsin and incubation at 37°C for 30 min in order to release virus from inclusions. Plates are incubated for 3-5 days at 37°C (5% CO₂) and observed for cytopathology typical of raccoon poxvirus. Titers are calculated as 50% endpoints based on cytopathology using the methods of Reed and Muench, The American Journal of Hygiene 27(3):493-497 (1938).

15

Example 2

PREPARATION OF VACCINE AND TESTING FOR EFFICACY IN CATS

1. Preparation of Master Seeds of RRPV-FIPV N and E1 Viruses

A single clone of each recombinant virus was selected for large-scale expansion to serve as a master seed virus. The criteria for selection were: 1) Demonstration of purity. Polymerase chain reaction was utilized to insure that the clone was uncontaminated with wild type virus. 2) Demonstration of adequate recombinant protein expression by Western blot or other antigen detection methods.

All recombinant virus expansions and titrations were done on Vero cells in MEM containing 2.5% FBS. Each plaque purified virus clone was expanded by inoculating a confluent monolayer of Vero cells in a 150 cm² flask (1×10^7 cells) with

This Sheet Has Been Replaced



- 13 -

1 ml of viral-cell lysate (approximately 10^7 infectious virus particles), and incubating at 37°C (5% CO_2) until 100% cytopathic effect was observed (2-3 days). This virus-cell lysate was titrated on Vero cells as described in Example 1, and served as a premaster seed virus stock to obtain the master seed virus. The MOI to be used to produce the highest titer master seed virus was determined by inoculating a confluent monolayer of Vero cells in a roller bottle (1×10^8 cells) with various MOIs of recombinant virus (e.g. 0.1, 0.05, 0.01, 0.005, and 0.001 $\text{TCID}_{50}/\text{cell}$.) The infected cells were incubated at 37°C until greater than 80% CPE was observed (approximately 3 days), and the titers of each infected roller bottle was determined. The master seed viruses were aliquoted into 1.5 ml ampules, which were sealed and stored in a liquid nitrogen freezer.

2. Preparation of Vaccines

3×10^7 Vero cells were seeded into 850 cm^2 roller bottles in 200 ml of growth media (MEM containing 0.5% lactalbumin hydrolysate and 5% FBS) and incubated for 18 hours at 37°C . The next day, the medium was removed from the cells and replaced with 50 ml of RRPV-FIPV N virus diluted to an MOI of 0.01 in infection media (MEM containing 0.5% lactalbumin hydrolysate and 2.5% FBS). The virus used was at the second passage beyond the master seed preparation. Virus was allowed to absorb to the cells for 30 min at 37°C , after which the volume of medium was adjusted to 150 ml per roller bottle. Roller bottles were incubated at 37°C until 100% cytopathology was evident (3 days). The virus-cell lysate was harvested and stored frozen (-70°C). The virus titer was determined to be $10^{6.97} \text{ TCID}_{50}/\text{ml}$.

RRPV-FIPV EI stocks were prepared in the same manner, except that an MOI of 0.1 was used. The final virus preparation was titered and found to contain $10^{6.5} \text{ TCID}_{50}/\text{ml}$. Wild type raccoon poxvirus was grown using the same methods as described above, and contained $10^{6.44} \text{ TCID}_{50}/\text{ml}$.

3. Vaccination

This Sheet Has Been Replaced



- 14 -

A group of twenty-four 9-month-old cats (specific pathogen-free, Harlan Sprague Dawley, Madison, WI), comprising seven males and seventeen females, was used to demonstrate the efficacy of the RRPV-FIPV N vaccine. Cats were divided into five groups and vaccinated twice, 21 days apart, as indicated below:

Group	# Cats	Vaccine	Volume (ml)	Viral Dose (TCID ₅₀)	Vaccination Route*
1	5	RRPV-FIPV N	3	10 ^{7.44}	SC
2	5	RRPV-FIPV N	1	10 ^{6.97}	IM
3	5	RRPV-FIPV N	3	10 ^{7.44}	ORAL
4	4	RRPV-FIPV N (1:10 Dilution)	3	10 ^{6.44}	SC
5	5	Wild Type RPV	3	10 ^{6.44}	SC

*SC = Subcutaneous

IM = Intramuscular

Oral = Oral

4. Challenge

Two weeks following the second vaccination, cats were orally inoculated with 10^{3.4} TCID₅₀ of Feline Enteric Coronavirus (strain 79-1683, ATCC VR-989). This virus induces a subclinical infection which can enhance subsequent FIPV infection. Three weeks later, cats were orally challenged with 10^{3.4} TCID₅₀ of FIPV (strain 79-1146, ATCC VR-990). Cats were monitored weekly for a total of 64 days after challenge for signs of clinical disease including: fever, icterus, leukopenia, anemia, weight loss, anorexia, depression, dehydration, and peritoneal swelling. Cats deemed moribund were euthanized by the attending veterinarian and post-mortem pathological examination was performed. Clinical disease signs were scored as follows:

This Sheet Has Been Replaced



- 15 -

	<u>SIGN</u>	<u>SCORE</u>
	Fever	103.0 - 103.9°F 1 point/day* 104.0 - 104.9°F 2 points/day ≥105.0°F 3 points/day
5	*For cats with baseline temperatures averaging 103°F, no points will be scored until temperatures are in excess of 1°F above baseline.	
	Dehydration	1 point/day
	Depression	1 point/day
	Anorexia	1 point/day
10	Peritoneal Swelling	1 point/day
	Icterus	1 point/day
	Weight Loss	>20% 1 point per observation >30% 2 points per observation >50% 5 points per observation
15	Leukopenia	decrease of 50% 3 points per observation counts <6000 2 points per observation
	Hematocrit	<25% PCV 3 points per observation
	Death	25 points

5. Evaluation of Induced Immunity to FIPV

20 Inoculation with virulent FIPV induced a fatal infection in 4/5 (80%) of the control cats, which were vaccinated with wild type raccoon poxvirus (Table 1). Both effusive and non-effusive forms of the disease were noted in the control cats. On the other hand, clinical disease was essentially absent after challenge of the subcutaneous vaccinates. The sporadic fever in these cats could be attributed to excitability and the

25 slight anemia on one day in cat 1264 is not a significant finding. The subcutaneous vaccinates showed a statistically significant reduction in clinical signs ($p < 0.05$, by ANOVA) and death ($p < 0.01$, by Chi Square Analysis) when compared to the control cats.

30 The intramuscular route of vaccination was less effective in that 2/5 (40%) of the cats succumbed to FIPV-induced disease. However, the onset of disease

This Sheet Has Been Replaced



- 16 -

5

in these cats was delayed when compared to the controls. The decreased efficacy may be related to the lower titer of virus inoculated into these cats because only a 1 ml dose could be administered by this route. There was also decreased efficacy when cats were inoculated by the oral route (60% mortality) which may indicate the need for a higher virus dose when vaccinated by this route.

The protection conferred against FIPV-caused disease by the subcutaneously administered vaccine was shown to be dose-dependent, confirming the benefit of a high-titer RRPV-FIPV vaccine in inducing protection against clinical disease induced by FIPV virus. A suitable vaccine dose contains viral antigen in the range of 10^4 - 10^8 TCID₅₀/ml, preferably 10^7 - 10^8 TCID₅₀/ml. A typical dose for administration to cats is 1-3 ml, and delivery by the subcutaneous route is preferred.

This Sheet Has Been Replaced



- 17 -

Table 1**TOTAL CLINICAL SCORES FOLLOWING CHALLENGE WITH FIPV**

CAT ID	Fever	Weight Loss	Leukopenia	Anemia	Chemical Signs	Death*	Total Score
SUBCUTANEOUS VACCINATES							
1260	0	0	0	0	0	0	0
1262	1	0	0	0	0	0	1
1264	1	0	0	0	0	0	4
1266	0	0	0	0	0	0	0
1268	2	0	0	0	0	0	2
INTRAMUSCULAR VACCINATES							
1270	13	6	0	0	33	25	77
1272	2	0	0	0	0	0	2
1297	23	0	0	0	34	25	81
1299	3	0	0	0	1	0	4
1301	3	0	0	0	0	0	3
ORAL VACCINATES							
1303	1	0	4	0	0	0	5
1305	0	1	0	0	5	0	6
1307	3	1	0	0	4	25	33
1309	2	1	6	0	8	25	40
1311	4	3	0	0	18	25	50
1/10 DOSE SUBCUTANEOUS VACCINATES							
1313	29	5	9	3	38	25	107
1316	17	0	0	0	20	25	62
1317	3	0	3	0	9	25	40
1319	1	0	0	0	6	0	7
CONTROLS							
1321	39	3	18	3	63	25	151
1323	5	0	2	0	14	25	46
1327	0	1	2	0	15	25	43
1329	5	1	6	0	11	25	48
1337	1	0	0	0	0	0	1

This Sheet Has Been Replaced

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Wasmoen, Terri
Chavez, Lloyd
Chu, Hsien-Jue
- (ii) TITLE OF INVENTION: Recombinant Raccoon Pox Viruse and Their
Use as an Effective Vaccine Against Infectious Peritonitis
Virus Disease
- (iii) NUMBER OF SEQUENCES: 4
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Darby & Darby PC
 - (B) STREET: 805 Third Avenue
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: US
 - (F) ZIP: 10022
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/125,516
 - (B) FILING DATE: 22-SEP-1993
 - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Schaffer, Robert
 - (B) REGISTRATION NUMBER: 31,194
 - (C) REFERENCE/DOCKET NUMBER: 9632/08669
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 212-527-7700
 - (B) TELEFAX: 212-753-6237
 - (C) TELEX: 236687

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 789 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

This Sheet Has Been Replaced



(vi) ORIGINAL SOURCE:

(A) ORGANISM: Feline infectious peritonitis virus

(vii) IMMEDIATE SOURCE:

(B) CLONE: FIPV E1

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

ATGAAGTACA TTTTGCTAAT ACTCGCGTGC ATAATTGCAT GCGTTTATGG TGAACGCTAC	60
TGTGCCATGC AAGACAGTGG CTTGCAGTGT ATTAATGGCA CAAATTCAAG ATGTCAAACC	120
TGCTTTGAAC GTGGTGATCT TATTTGCCAT CTTGCTAACT GGAACCTCAG CTGGTCTGTA	180
ATATTGATTG TTTTATAAC AGTGTTACAA TATGCCAGAC CACAATTTAG CTGGCTCGTT	240
TATGGCATTG AAATGCTGAT CATGTGGCTA TTATGGCCTA TTGTTCTAGC GCTTAOGATT	300
TTTAATGCAT ACTCTGAGTA CCAAGTTTCC AGATATGTAA TGTTCGGCTT TAGTGTTCGA	360
GGTGCACTTG TAACGTTTGC ACTTTGGATG ATGTATTTTG TGAGATCTGT TCAGCTATAT	420
AGAAGAACCA AATCATGGTG GTCTTTTAAT CCTGAGACTA ATGCAATTCT TTGTGTTAAT	480
GCATTGGGTA GAAGTTATGT GCTTCCCTTA GATGGTACTC CTACAGGTGT TACCCTTACT	540
CTACTTTCAG GAAATCTATA TGCTGAAGGT TTCAAAATGG CTGGTGGTTT AACCATOGAG	600
CATTTCCTTA AATACGTCAT GATTGCTACA CCTAGTAGAA CCATCGTTTA TACATTAGTT	660
GGAAAACAAT TAAAAGCAAC TACTGCCACA GGATGGGCTT ACTAOGTAAA ATCTAAAGCT	720
GGTGATTACT CAACAGAAGC ACGTACTGAC AATTGAGTG AACATGAAAA ATTATTACAT	780
ATGGTGTA	789

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1134 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Feline infectious peritonitis virus

(vii) IMMEDIATE SOURCE:

(B) CLONE: FIPV N

This Sheet Has Been Replaced



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

ATGGCCACAC AGGGACAACG CGTCAACTGG GGAGATGAAC CTTCCAAAAG ACGTGGTGGT	60
TCTAACTCTC GTGGTCGGAA GAATAATGAT ATACCTTTGT CATTCTACAA CCCATTACC	120
CTCGAACAAG GATCTAAATT TTGGAATTTA TGTCCGAGAG ACCTTGTTCC CAAAGGAATA	180
GGTAATAAGG ATCAACAAAT TGGTTATTGG AATAGACAGA TTCGTTATCG TATTGTAAAA	240
GGCCAGOGTA AGGAACTCGC TGAGAGGTGG TTCTTTTACT TCTTAGGTAC AGGACCTCAT	300
GCTGATGCTA AATTCAAAGA CAAGATTGAT GGAGTCTTCT GGGTTGCAAG GGATGGTGCC	360
ATGAACAAGC CCACAACGCT TGGCACTCGT GGAACCAATA ACGAATCCAA ACCACTGAGA	420
TTTGATGGTA AGATACCGCC ACAGTTTCAG CTGGAAGTGA ACOGTTCTAG GAACAATTCA	480
AGGTCTGGTT CTCAGTCTAG ATCTGTTTCA AGAAACAGAT CTCAATCTAG AGGAAGACAC	540
CATTCCAATA ACCAGAATAA TAATGTTGAG GATACAATTG TAGCGTGCT TGAAAAATTA	600
GGTGTACTG ACAAAACAAG GTCACGTTCT AAACCTAGAG AACGTAGTGA TTCCAAACCT	660
AGGGACACAA CACCTAAGAA TGCCAACAAA CACACCTGGA AGAAAACTGC AGGCAAGGGA	720
GATGTGACAA CTTTCTATGG TGCTAGAAGT AGTTCAGCTA ACTTTGGTGA TAGTGATCTC	780
GTGCCAATG GTAACGCTGC CAAATGCTAC CCTCAGATAG CTGAATGTGT TCCATCAGTG	840
TCTAGCATAA TCTTTGGCAG TCAATGGTCT GCTGAAGAAG CTGGTGATCA AGTGAAAGTC	900
ACGCTCACTC ACACCTACTA CCTGCCAAAG GATGATGCCA AACTAGTCA ATTCCTAGAA	960
CAGATTGACG CTTACAAGCG ACCTTCTGAA GTGGCTAAGG ATCAGAGGCA AAGAAGATCC	1020
CGTTCTAAGT CTGCTGATAA GAAGCCTGAG GAGTTGTCTG TAACTCTTGT GGAGGCATAC	1080
ACAGATGTGT TTGATGACAC ACAGGTTGAG ATGATTGATG AGGTTACGAA CTAA	1134

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8710 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline infectious peritonitis virus

(vii) IMMEDIATE SOURCE:

This Sheet Has Been Replaced



(B) CLONE: psc11f1

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

CGAAGGGGCC	TCGTGATAAG	CCTATTTTTA	TAGGTTAATG	TCATGATAAT	AATGGTTTCT	60
TAGACGTCAG	GTTGGCATT	TGGGGGAAAT	GTGCGCGGAA	CCCTATTTTG	TTTATTTTTC	120
TAAATACATT	CAATATATGA	TCOGCTCATG	AGACATAAAC	CCTGATAAAT	GCTTCAATAA	180
TATTGAAAAA	GGABGGATAT	GAGTATTCAA	CATTTCGGTG	TGCGCCCTAT	TCCCTTTTTF	240
GCGGCATTTT	GCCTTCCTGT	TTTGTCTCAC	CCAGAAAGCG	TGGTGAAAGT	AAAAGATGCT	300
GAAGATCAAT	TGGGTGCAAG	AGTGGGTTAC	ATCGAACTGG	ATCTCAACAG	CGGTAAAGATC	360
CTTGAGAGTT	TTGCGCCCGA	AGAACGTTTT	CCAATGATGA	GCACTTTTAA	AGTTCTGCTA	420
TGTGGGCGGG	TATTATCCCG	TATTGACGCC	GGGCAAGAGC	AACTCGGTGG	CGGCATACAC	480
TATCTCAGA	ATGACTTGGT	TGAGTACTCA	CCAGTCACAG	AAAAGCATCT	TACGGATGGC	540
ATGACAGTAA	GAGAATTATG	CAGTCTTGCC	ATAACCATGA	GTGATACAC	TGCGGCCAAC	600
TTACTTCTGA	CAACGATCGG	AGGACCGAAG	GAGCTAACCG	CTTTTTTGCA	CAACATGGGG	660
GATCATGTAA	CTCGCCTTGA	TGTTGGGGAA	CCGGAGCTGA	ATGAAGCCAT	ACCAACCGAC	720
GAGCGTGACA	CCACGATGCC	TGTAGCAATG	GCAACAAAGT	TGCGCAAACT	ATTAAGTGGC	780
GAACCTACTA	CTCTAGCTTC	CGGCCAACAA	TTAATAGACT	GGATGGAGGC	GGATAAAGTT	840
GCAGGACCAC	TTCTGCGCTC	GGCCCTTCGG	GCTGGCTGGT	TTATGTCTGA	TAAATCTGGA	900
GCGCGTGAGC	GTGGGTCTCG	CGGTATCATT	GCAGCACTGG	GGCCAGATGG	TAAGCCCTCC	960
CGTATCGTAG	TTATCTACAC	GACGGGGAGT	CAGGCAACTA	TGGATGAACG	AAATAGACAG	1020
ATCGCTGAGA	TAGGTGCTTC	ACTGATTAAAG	CATTGGTAAC	TGTCAGACCA	AGTTTACTCA	1080
TATATACTTT	AGATTGATTT	AARACTTCAT	TTTTAATTTA	AAAGCATCTA	GGTGAAGATC	1140
CTTTTGTATA	ATCTCATGAC	CAAAATCCCT	TACGTGAGT	TTTGGTTCCA	CTGAGCGTCA	1200
GACCCCGTAG	AAAAGATCAA	AGGATCTTCT	TGAGATCCTT	TTTTCTCGCG	CGTAATCTGC	1260
TGCTTGCAAA	CAAAAAAACC	ACCGCTACCA	GCGGTGGTTT	GTTTGCCTGA	TCAAGAGCTA	1320
CCAACCTCTT	TTCGAAAGGT	AACTGGCTTC	AGCAGAGCGC	AGATAACCAA	TACTGTCTCT	1380
CTAGTGTAGC	CGTAGTAAAG	CAACCACTTC	AAGAAGCTCG	TAGCACCGCC	TACATACCTC	1440
GCTCTGCTAA	TCTGTATACC	AGTGGCTGCT	GCCAGTGGCG	ATAAGTCTGT	TCTTACGGGG	1500

This Sheet Has Been Replaced



TTGGACTCAA	GACGATAGTT	ACCGGATAAG	GCGCAGCGGT	CGGGCTGAAC	GGGGGGTTGG	1560
TGCACACAGC	CCAGCTTGGA	GCGAAGSACC	TACACOGAAC	TGAGATACCT	ACAGCGTGAG	1620
CATTGAGAAA	GCGCCAGGCT	TCCOGAAGGG	AGAAAGGOGG	ACAGGTATCC	GGTAAGCGGC	1680
AGGGTCGGAA	CAGGAGAGCG	CACGAGGGAG	CTTCCAGGGG	GAAAGCGCTG	GTATCTTAT	1740
AGTCCTGTGG	GGTTTGGCCA	CCCTGACTT	GAGCGTCGAT	TTTGTGTATG	CTCGTCAGGG	1800
GGGCGGAGCC	TATGGAAAAA	CGCCAGCAAC	GCGGCCTTTT	TACGGTTGCT	GGCCTTTTGC	1860
TGGCCTTTTG	CTCACATGTT	CTTCTCTGGG	TTATCCCCTG	ATTCTGTGGA	TAACCGTATT	1920
ACCGCCTTTG	AGTGAGCTGA	TACCGCTGGC	CGCAGCCGAA	CGACCGAGCG	CAGCGAGTCA	1980
GTGAGCGAGG	AAGCGGAAGA	GCGCCCAATA	CGCAAACCGC	CTCTCCCGGC	GCGTTGGCCG	2040
ATTCATTAA	GCGCTGGCA	CGACAGGTTT	CCCGACTGGA	AAGCGGGCAG	TGAGCGCAAC	2100
GCAATTAA	TGAGTTAGCT	CATCTATTAG	GCACCCAGG	CTTACACTT	TATGCTTCGG	2160
GCTCGTATGT	TGTGTGGAAT	TGTGAGGGGA	TAACAATTTC	ACACAGGAAA	CAGCTATGAC	2220
CATGATTAGG	CCAAGCTTTT	GCGATCAATA	AATGGATCAC	AACCAGTATC	TCTTACGAT	2280
GTTCTTGGCA	GATGATGATT	CATTTTAA	GTATTTGGCT	AGTCAAGATG	ATGAAATCTT	2340
CATTATCTGA	TATATTGCA	ATCACTCAAT	ATCTAGACTT	TCTGTTATTA	TTATTGATCC	2400
AATCAAAAAA	TAAATTAGAA	GCGTGGGTC	ATTGTTATGA	ATCTCTTTCA	GAGGAATACA	2460
GACAATTGAC	AAAATTACAA	GACTTTCAAG	ATTTTAAAAA	ACTGTTTAA	AAGGTCCCTA	2520
TGTTACAGA	TGGAAGGGTC	AACTTAATA	AAGGATATTT	GTTGACTTT	GTGATTAGTT	2580
TGATGCGATT	CAAAAAGAA	TCCTCTCTAG	CTACCACCGC	AATAGATGCT	GTTAGATACA	2640
TAGATCCTCG	TGCAATATC	GCATTTTCTA	ACGTGATGGA	TATATTAAAG	TGAAATAAAG	2700
TGAACAATAA	TTAATTCTTT	ATTGTCATCA	TGAACGGGGG	ACATATTCAG	TTGATAATCG	2760
GCCCCATGTT	TTCAGGTAAA	AGTACAGAAT	TAATTAGACG	AGTTAGACGT	TATCAATAG	2820
CTCAATATAA	ATGCGTGACT	ATAAAATATT	CTAAGATATA	TAGATAAGGA	ACGGGACTAT	2880
GGACGCATGA	TAAGAATAAT	TTTGAAGCAT	TGGAAGCAAC	TAAACTATGT	GATCTCTTGG	2940
AATCAATTAC	AGATTCTCTC	GTGATAGGTA	TGATGAAGG	ACAGTTCTTT	CCAGACATTG	3000
TGAATTCCG	AGCTTGGCTG	CAGGTGGGGG	ATCCCCCTCG	CCCGGTTATT	ATTATTTTGG	3060
ACACCAGACC	AACTGGTAAT	GGTAGCGAAC	GCGCTCAGC	TGAATTCCGC	CGATACTGAC	3120

This Sheet Has Been Replaced



GGGCTCCAGG	AGTOGTGCGC	ACCAATCCCC	ATATGGAAAC	CGTOGATATT	CAGCCATGTG	3180
CCTTCTTCOG	CGTGCAGCAG	ATGGCGATGG	CTGGTTTCCA	TCAGTIGCTG	TTGACTGTAG	3240
CGGCTGATGT	TGAACTGGAA	GTCGCCGCGC	CACTGGTGTG	GGCCATAATT	CAATTGCGGC	3300
GTCCOGCAGC	GCAGACCGTT	TTCGCTCGGG	AAGACGTACG	GGGTATACAT	GTCTGACAAT	3360
GGCAGATCCC	AGCGGTCAAA	ACAGGCGGCA	GTAAGGCGGT	CGGGATAGTT	TTCTTGCGGC	3420
CCTAATCOGA	GCCAGTTTAC	COGCTCTGCT	ACCTGCGCCA	GCTGGCAGTT	CAGGCCAATC	3480
CGCGCOGGAT	GCGGTGTATC	GCTCGCCACT	TCAACATCAA	CGGTAATCGC	CATTTGACCA	3540
CTACCATCAA	TCCGGTAGGT	TTTCCGGCTG	ATAAATAAGG	TTTTCCCCTG	ATGCTGCCAC	3600
GCGTGACOGG	TCGTAATCAG	CACCGCATCA	GCAAGTGTAT	CTGCCGTGCA	CTGCAACAAC	3660
GCTGCTTCGG	CCTGGTAAATG	GCCGCGCGCC	TTCCAGCGTT	CGACCCAGGC	GTTAGGGTCA	3720
ATGCGGGTCG	CTTCACTTAC	GCCAATGTG	TTATCCAGCG	GTGCAOGGGT	GAACTGATCG	3780
CGCAGCGGOG	TCAGCAGTTG	TTTTTTATCG	CCAATCCACA	TCTGTGAAAG	AAAGCCTGAC	3840
TGGCGGTTAA	ATTGCCAACG	CTTATTACCC	AGCTOGATGC	AAAAATCCAT	TTGCTGGTG	3900
GTCAGATGOG	GGATGGCGTG	GGACGCGGCG	GGGAGCGTCA	CACTGAGGTT	TTGCGCCAGA	3960
CGCCACTGCT	GCCAGGCGCT	GATGTGCCOG	GCTTCTGACC	ATGCGGTGCG	GTTGGGTGTC	4020
ACTACGOGTA	CTGTGAGCCA	GAGTTGCCCG	GCGCTCTCCG	GCTGCGGTAG	TTGAGGCAGT	4080
TCAATCAACT	GTTTACCTTG	TGGAGOGACA	TCCAGAGGCA	CTTCACCGCT	TGCCAGCGGC	4140
TTACCATCCA	GCGCCACCAT	CCAGTGCAGG	AGCTCGTTAT	CGCTATGACG	GAACAGGTAT	4200
TCGCTGGTCA	CTTCGATGGT	TTGCCCGGAT	AAACGGAACT	GGAAAAACTG	CTGCTGGTGT	4260
TTTGCTTCCG	TCAGCGCTGG	ATGCGGCGTG	CGGTGGCAA	AGACCAGACC	GTTTCATACAG	4320
AACTGGCGAT	CGTTGCGCGT	ATCGCCAAAA	TCACGCGCGT	AAGCCGACCA	CGGGTTGCCG	4380
TTTTCATCAT	ATTTAATCAG	CGACTGATCC	ACCCAGTCCC	AGAOGAAGCC	GCCCTGTAAA	4440
CGGGGATACT	GACGAAACGC	CTGCCAGTAT	TTAGCGAAAC	CGCCAAGACT	GTTACCCATC	4500
GCGTGCGCGT	ATTCGCAAAG	GATCAGCGGG	CGCGTCTCTC	CAGGTAGCGA	AAGCCATTTT	4560
TTGATGGACC	ATTTGCGCAC	AGCGGGGAAG	GGCTGGTCTT	CATCCACGCG	CGCGTACATC	4620
GGGCAAATAA	TATCGGTGGC	CGTGGTGTG	GCTCCGCGCG	CTTCATACTG	CACCGGGGCG	4680
GAAGGATCGA	CAGATTTGAT	CCAGCGATAC	AGCGCGTCGT	GATTAGCGCC	GTGGCCTGAT	4740

This Sheet Has Been Replaced



TCATTCCCCA	GCGACCAGAT	GATCACACTC	GGGTGATTAC	GATOGGCGTG	CACCATTOGC	4800
GTACGCGTT	CGCTCATGCG	CGGTAGCCAG	CGCGGATCAT	CGGTACAGCG	ATTGATTGGC	4860
ACCATGCCGT	GGGTTTCAAT	ATTGGCTTCA	TCCACCACAT	ACAGGCGGTA	GCGGTGCGAC	4920
AGCGTGATCC	ACAGGGGATG	GTTOGGATAA	TGCGAACAGC	GCAOGGCGTT	AAAGTTGTTC	4980
TGCTTCATCA	GCAGGATATC	CTGCACCATC	GTCTGCTCAT	CCATGACCTG	ACCATGCAGA	5040
GGATGATGCT	CGTGACGGTT	AAOGCCTCGA	ATCAGCAACG	GCTTGCGGTT	CAGCAGCAGC	5100
AGACCATTTT	CAATCCGCAC	CTOGCGGAAA	COGACATGCG	AGGCTTCTGC	TTCAATCAGC	5160
GTGCCGTGGG	CGGTGTGCAG	TTCAACCACC	GCACGATAGA	GATTOGGGAT	TTGGGCGCTC	5220
CACAGTTTOG	GGTTTTOGAC	CTTGAGAGGT	AGTGTGACGC	GATCGGCATA	ACCACCACGC	5280
TCATCGATAA	TTTCACCGCC	GAAAGGCGCG	GTGCGGCTGG	CGACCTGCGT	TTACCCCTGC	5340
CATAAAGAAA	CTGTTACCCG	TAGGTAGTCA	CGCAACTGCG	CGCACATCTG	AACTTCAGCC	5400
TCCAGTACAG	CGCGGCTGAA	ATCATCATT	AAGCGAGTGG	CAACATGGAA	ATCGCTGATT	5460
TGTGTAGTCG	GTTTATGCAG	CAACGAGACG	TCACGGAAAA	TGCGGCTCAT	CGGCCACATA	5520
TCTGATCTT	CCAGATAACT	GCGGTCACCT	CAAOGCAGCA	CCATCACCGC	GAGGOGGTTT	5580
TCTCGGCGCG	GTAAAAATGC	GCTCAGGTCA	AATTGAGACG	GCAACGACT	GTCTGGCGCG	5640
TAACCGACCC	AGCGCCCGTT	GCACCACAGA	TGAAACGCGG	AGTTAACGCC	ATCAAAATA	5700
ATTOGOGTCT	GGCCTTCCTG	TAGCCAGCTT	TCATCAACAT	TAAATGTGAG	CGAGTAACAA	5760
CCCGTOGGAT	TCTCGTGSGG	AACBAAOCGC	GGATTGACCG	TAAATGGGATA	GGTTACGTTG	5820
GGTAGATGG	GCGCATCGTA	ACCGTGCACT	TGCCAGTTTG	AGGGGACGAC	GACAGTATCG	5880
GCCTCAGGAA	GATOGCACTC	CAGCCAGCTT	TCCGGCACCG	CTTCTGGTGC	CGGAAACCG	5940
GCAAAGCGCC	ATTOGCCATT	CAGGCTGCGC	AACGTGTTGG	AAGGGCGATC	GGTGCGGGCC	6000
TCTTCGCTAT	TACGCCAGCT	GGCGAABGGG	GGATGTGCTG	CAAGGCGATT	AAATGGGTA	6060
ACGCCAGGGT	TTTCCAGTGC	ACGAGGTTGT	AAAACGACGG	GATCCCTCGA	GGAATTCATT	6120
TATAGCATAG	AAAAAAACAA	AATGAAATTC	TACTATATTT	TTACATACAT	ATATTCTAAA	6180
TATGAAAGTG	GTGATTGTGA	CTAGCGTAGC	ATCGCTTCTA	GACATATACT	ATATAGTAAT	6240
ACCAATACTC	AAGACTACGA	AACGTATACA	ATCTCTTATC	ATGTGGGTAA	TGTTCTOGAT	6300
GTCGAATAGC	CATATGCCGG	TAGTTGCGAT	ATACATAAAC	TGATCACTAA	TTCCAAACCC	6360

This Sheet Has Been Replaced



ACCCGCTTTT	TATAGTAACT	TTTTACCCCA	TAAATAATAA	ATACAATAAT	TAATTTCTCG	6420
TAAAAGTAGA	AAATATATTC	TAATTTATTG	CACGGTAAGG	AAGTAGAATC	ATAAAGAACA	6480
GTGAOGGATC	CCAATTCGGG	CATTTTGGT	TTGAACTAAA	CAAAATGAAG	TACATTTTGC	6540
TAATACTCGC	GTCATAAAT	GCATGCGTTT	ATGGTGAACG	CTACTGTGCC	ATGCAAGACA	6600
GTGGCTTGCA	GTGTATTAAT	GGCACAAATT	CAAGATGTCA	AACCTGCTTT	GAACGTGGTG	6660
ATCTTATTGG	GCATCTTGCT	AACTGGAAT	TCAGCTGGTC	TGTAATATTG	ATTGTTTTTA	6720
TAACAGTGTT	ACAATATGGC	AGACCACAAT	TTAGCTGGCT	CGTTTATGGC	ATTAAAATGC	6780
TGATCATGTG	GCTATTATGG	CCTATTGTTT	TAGCGCTTAC	GATTTTTAAT	GCATACTCTG	6840
AGTACCAAGT	TTCCAGATAT	GTAATGTTTG	GCTTTAGTGT	TGCAGGTGCA	GTTGTAAAGT	6900
TTGCACTTTG	GATGATGTAT	TTGTGAGAT	CTGTTTACCT	ATATAGAAGA	ACCAAATCAT	6960
GGTGGTCTTT	TAATCCTGAG	ACTAATGCAA	TTCTTTGTGT	TAATGCATTG	GGTAGAAGTT	7020
ATGTGCTTCC	CTTAGATGGT	ACTCCTACAG	GTGTTACCTT	TACTCTACTT	TCAGGAAATC	7080
TATATGCTGA	AGGTTTCAAA	ATGGCTGGTG	GTTTAACCAT	CGAGCATTTG	CCTAABATACG	7140
TCATGATTGC	TACACCTAGT	AGAACCATCG	TTTATACATT	AGTTGGAAAA	CAATTAAAAG	7200
CAACTACTGC	CACAGGATGG	GCTTACTACG	TAAATCTTAA	AGCTGGTGAT	TACTCAACAG	7260
AAGCAGGTAC	TGACAATTTG	AGTGAACATG	AAAAATTATT	ACATATGGTG	TAACTAAACT	7320
TTCAAATGGG	GGAATTTCTG	GAGCGTATGG	CAAAOGAAGG	AAAAATTAGT	TATAGTAGCC	7380
GCACTCGATG	GGACATTTCA	ACGTAAACCG	TTTAATAATA	TTTGAATCT	TATTCCATTA	7440
TCTGAAATGG	TGGTAAAACT	AACTGCTGTG	TGTATGAAAT	GCTTTAAGGA	GGCTTCCTTT	7500
TCTAAACGAT	TGGGTGAGGA	AACCGAGATA	GAAATAATAG	GAGGTAATGA	TATGTATCAA	7560
TCGGTGTGTA	GAAAGTGTTA	CATCGACTCA	TAATATTATA	TTTTTTATCT	AAAAAACTAA	7620
AAATAAACAT	TGATTAAATT	TTAATATAAT	ACTTAAAAAT	GGATGTTGTG	TCGTTAGATA	7680
AACCGTTTAT	GTATTTTGAG	GAAATTGATA	ATGAGTTAGA	TTACGAACCA	GAAAGTGCAA	7740
ATGAGGTCCG	AAAAAAACTG	CCGTATCAAG	GACAGTTAAA	ACTATTACTA	GGAGAATTAT	7800
TTTTTCTTAG	TAAGTTACAG	CGACACGGTA	TATTAGATGG	TGCCACCGTA	GTGTATATAG	7860
GATCTGCTCC	CGGTACACAT	ATACGTTATT	TGAGAGATCA	TTTCTATAAT	TTAGGAGTGA	7920
TCATCAAATG	GATGCTAATT	GACGGCCGCC	ATCATGATCC	TATTTTAAAT	GGATTGCGTG	7980

This Sheet Has Been Replaced



ATGTGACTCT AGTGACTCGG TTCGTTGATG AGGAATATCT ACGATCCATC AAAAAACAAC	8040
TGCATCCTTC TAAGATTATT TTAATTTCTG ATGTGAGATC CAAACGAGGA GGAAATGAAC	8100
CTAGTACGGC GGATTACTA AGTAATTACG CTCTACAAA TGTGATGATT AGTATTTTAA	8160
ACCCCGTGGC GTCTAGTCTT AAATGGAGAT GCCCGTTTCC AGATCAATGG ATCAAGGACT	8220
TTTATATCCC ACACGGTAAT AAAATGTTAC AACCTTTTGC TCCTTCATAT TCAGGGCCGT	8280
CGTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC	8340
ACATCCCCCT TTOGCCAGCT GCGGTAATAG CGAAGAGGCC OGCACOGATC GCCCTTCCCA	8400
ACAGTTGCGC AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCT TTACGCATCT	8460
GTGCGGTATT TCACACOGCA TATGGTGCAC TCTCAGTACC ATCTGCTCTG ATGCOGCATA	8520
GTTAAGCCAG TACACTCOGC TATOGCTACG TGACTGGGTC ATGGCTGCGC CCGACACCC	8580
GCCAACACCC GCTGACGCGC CCTGACGGGC TTGTCTGCTC COGGCATCCG CTTACAGACA	8640
AGCTGTGACC GTCTCCGGGA GCTGCAATGT TCAGAGGTTT TCACCGTCAT CACCGAAACG	8700
CGCGAGGCAG	8710

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9019 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus

(vii) IMMEDIATE SOURCE:

- (B) CLONE: psc11e1

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

GAAAGGGCCT CGTGATAQGC CTATTTTAT AGGTTAATGT CATGATAATA ATGGTTTCTT	60
AGACGTCAGG TGGCACITTT CGGGGAAATG TGCGCGGAAC CCCTATTGT TTATTTTCT	120
AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT	180
ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTTCGTGT CGCCCTTATT CCCTTTTGTG	240
CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG	300

This Sheet Has Been Replaced



AAGATCAGTT	GGGTGCACGA	GTGGGTTACA	TCGAAC TGGA	TCTCAACAGC	GGTAAGATCC	360
TTGAGAGTTT	TCGCCCCGAA	GAACGTTTTC	CAATGATGAG	CACTTTTAAA	GTTCTGCTAT	420
GTGGCGGGT	ATTATCCOGT	ATTGACGCCG	GGCAAGAGCA	ACTCGGTGCG	CGCATACACT	480
ATTCTCAGAA	TGACTTGGTT	GAGTACTCAC	CAGTCACAGA	AAAGCATCTT	ACGGATGGCA	540
TGACAGTAAG	AGAATTATGC	AGTGCTGCCA	TAACCATGAG	TGATAACACT	GCGGCCAACT	600
TACTTCTGAC	AACGATCGGA	GGACOGAAGG	AGCTAACCGC	TTTTTTGCAC	AACATGGGGG	660
ATCATGTAAC	TCGCCTTGAT	CGTTGGGAAC	CGGAGCTGAA	TGAAGCCATA	CCAAACGACG	720
AGCGTGACAC	CACGATGCCT	GTAGCAATGG	CAACAACGTT	GCGCAAACTA	TTAACTGGCG	780
AACTACTTAC	TCTAGCTTCC	CGGCAACAAT	TAATAGACTG	GATGGAGGCG	GATAAAGTTG	840
CAGGACCACT	TCTGCGCTCG	GCCCTTCGGG	CTGGCTGGTT	TATTGCTGAT	AAATCTGGAG	900
CCGGTGAGCG	TGGGTCTGCG	GGTATCATTG	CAGCACTGGG	GCCAGATGGT	AAGCCCTCCC	960
GATCGTAGT	TATCTACACG	ACGGGGAGTC	AGGCAACTAT	GGATGAACGA	AATAGACAGA	1020
TCGCTGAGAT	AGGTGCCTCA	CTGATTAAAGC	ATTGGTAACT	GTCAGACCAA	GTTTACTCAT	1080
ATATACTTTA	GATTGATTTA	AAACTTCATT	TTTAATTTAA	AAGGATCTAG	GTGAAGATCC	1140
TTTTTGATAA	TCTCATGACC	AAAATCCCTT	AACGTGAGTT	TTCGTTCCAC	TGAGCGTCAG	1200
ACCCCGTAGA	AAAGATCAAA	GGATCTTCTT	GAGATCCTTT	TTTTCTGCGC	GTAATCTGCT	1260
GCTTGCAAAC	AAAAAAACCA	CGCTACCAG	CGGTGGTTTG	TTTGCCGGAT	CAAGAGCTAC	1320
CAACTCTTTT	TCCGAAGGTA	ACTGGCTTCA	GCAGAGCGCA	GATACCAAAT	ACTGTCCTTC	1380
TAGTGTAGCC	GTAGTTAGGC	CACCACTTCA	AGAACTCTGT	AGCACCGCCT	ACATACCTCG	1440
CTCTGCTAAT	CCTGTTACCA	GTGGCTGCTG	CCAGTGGCGA	TAAGTGGTGT	CTTACCGGGT	1500
TGGACTCAAG	ACGATAGTTA	COGATAAGG	CGCAGCGGTC	GGGCTGAACG	GGGGGTTCGT	1560
GCACACAGCC	CAGCTTGGAG	CGAACGACCT	ACACCGAACT	GAGATACCTA	CAGCGTGAGC	1620
ATTGAGAAAG	CGCCACGCTT	CCCGAAGGGA	GAAAGGCGGA	CAGGTATCCG	GTAAGCGGCA	1680
GGGTGGAAC	AGGAGAGCGC	ACGAGGGAGC	TTCCAGGGGG	AAACGCCCTGG	TATCTTTATA	1740
GTCTGTGCGG	GTTTCGCCAC	CTCTGACTTG	AGCGTCGATT	TTGTGATGC	TCGTCAGGGG	1800
GGCGGAGCCT	ATGGAAAAAC	GCCAGCAACG	CGGCCTTTTT	ACGGTTCCTG	GCCTTTTGCT	1860
GGCCTTTTGC	TCACATGTTC	TTTCTGCGT	TATCCCCTGA	TTCTGTGGAT	AACCGTATTA	1920

This Sheet Has Been Replaced



COGCCCTTTGA	GTGAGCTGAT	ACCGCTCGCC	GCAGCCGAAC	GACCGAGCGC	AGCGAGTCAG	1980
TGAGCGAGGA	AGCGGAAGAG	CGCCCAATAC	GCBAACCGCC	TCTCCCCGCG	CGTTGGCOGA	2040
TTCAATTAATG	CAGCTGGCAC	GACAGGTTTC	COGACTGGAA	AGCGGGCAGT	GAGCGCAACG	2100
CAATTAATGT	GAGTTAGCTC	ACTCATTAGG	CACCCCAGGC	TTTACACTTT	ATGCTTCOGG	2160
CTCGTATGTT	GTGTGGAATT	GTGAGCGGAT	AACAATTTCA	CACAGGAAAC	AGCTATGACC	2220
ATGATTACGC	CAAGCTTTTG	CGATCAATAA	ATGGATCACA	ACCAGTATCT	CTTAACGATG	2280
TTCTTCGCAG	ATGATGATTC	ATTTTPTAAG	TATTTGGCTA	GTCAAGATGA	TGAAATCTTC	2340
ATTATCTGAT	ATATTGCAAA	TCACTCAATA	TCTAGACTTT	CTGTTATTAT	TATTGATCCA	2400
ATCAAAAAAT	AAATTAGAAG	COGTGGGTCA	TTGTTATGAA	TCTCTTTCAG	AGGAATACAG	2460
ACAATTGACA	AAATTCACAG	ACTTTCAAGA	TTTTAAAAAA	CTGTTTAAAC	AGGTCCCTAT	2520
TGTTACAGAT	GGAAGGGTCA	AACTTAATAA	AGGATATTTC	TTCGACTTTG	TGATTAGTTT	2580
GATGCGATTC	AAAAAGAAT	CCTCTCTAGC	TACCACCGCA	ATAGATCCTG	TTAGATACAT	2640
AGATCCTCGT	CGCAATATCG	CATTTTCTAA	CGTGATGGAT	ATATTAAAGT	CGAATAAAGT	2700
GAACAATAAT	TAATTCTTTA	TTGTCATCAT	GAACGGCGGA	CATATTCAGT	TGATAATCGG	2760
CCCCATGTTT	TCAGGTAAAA	GTACAGAATT	AATTAGAOGA	GTTAGACGTT	ATCAATAGC	2820
TCAATATAAA	TGCGTGACTA	TAAAATATTC	TAACGATAAT	AGATACGGAA	CGGGACTATG	2880
GACGCATGAT	AAGAATAATT	TTGAAGCATT	GGAAGCAACT	AAACTATGTG	ATCTCTTGGA	2940
ATCAATTACA	GATTTCTCOG	TGATAGGTAT	CGATGAAGGA	CAGTTCCTTC	CAGACATTGT	3000
TGAATTCCGA	GCTTGGCTGC	AGGTGCGGGA	TCCCCCTGCG	CCGGTTATTA	TTATTTTTGA	3060
CACCAGACCA	ACTGGTAATG	GTAGCGAACG	GCGCTCAGCT	GAATTCOGCC	GATACTGAOC	3120
GGCTCCAGGA	GTOFTCGCCA	CCAATCCCCA	TATGGAAACC	GTCGATATTC	AGCCATGTGC	3180
CTTCTTCOGC	GTGCAGCAGA	TGGCGATGGC	TGGTTTCCAT	CAGTTGCTGT	TGACTGTAGC	3240
GGCTGATGTT	GAACTGGAAG	TGCGCGCGCC	ACTGGTGTGG	GCCATAATTC	AATTCGCGCG	3300
TCCCGCAGOG	CAGACCGTTT	TCGCTCGGGA	AGACGTACGG	GGTATACATG	TCTGACAATG	3360
GCAGATCCCA	GCGGTCAAAA	CAGGCGGCAG	TAAGGCGGTC	GGGATAGTTT	TCTTGCGGCC	3420
CTAATCCGAG	CCAGTTTACC	CGCTCTGCTA	CCTGCGCCAG	CTGGCAGTTC	AGGCCAATCC	3480
GCGCOGGATG	CGGTGTATCG	CTCGCCACTT	CAACATCAAC	GGTAATCGCC	ATTTGACCAC	3540

This Sheet Has Been Replaced



TACCATCAAT	COGGTAGGTT	TTCOGGCTGA	TAAATAAGGT	TTTCCCCTGA	TGCTGCCACG	3600
CGTGACCGGT	CGTAATCAGC	ACCGCATCAG	CAAGTGTATC	TGCCGTGCAC	TGCAACAACG	3660
CTGCTTCGGC	CTGGTAATGG	CCCGCCGCCT	TCCAGCGTTC	GACCCAGGCG	TTAGGGTCAA	3720
TGCGGGTCGC	TTCACTTACG	CCAATGTCGT	TATCCAGCGG	TGCACGGGTG	AACTGATCGC	3780
GCAGCGGCGT	CAGCAGTTGT	TTTTTATCGC	CAATCCACAT	CTGTGAAAGA	AAGCCTGACT	3840
GGCGGTTAAA	TTGCCAACGC	TTATTACCCA	GCTCGATGCA	AAAATCCATT	TCGCTGGTGG	3900
TCAGATGCGG	GATGGCGTGG	GACGCGGCGG	GGAGCGTCAC	ACTGAGGTTT	TCOGCCAGAC	3960
GCCACTGCTG	CCAGGCGCTG	ATGTGCCCGG	CTTCTGACCA	TGCGGTGCGG	TTCGGTTGCA	4020
CTACGCGTAC	TGTGAGCCAG	AGTTGCCCGG	CGCTCTCCGG	CTGCGGTAGT	TCAGGCAGTT	4080
CAATCAACTG	TTTACCTTGT	GGAGCGACAT	CCAGAGGCAC	TTACCGCTT	GCCAGCGGCT	4140
TACCATCCAG	CGCCACCATC	CAGTGCAGGA	GCTCGTTATC	GCTATGACGG	AACAGGTATT	4200
CGCTGGTCAC	TTCGATGGTT	TGCCCGGATA	AACGGAACTG	GAAAAACTGC	TGCTGGTGT	4260
TTGCTTCGGT	CAGCGCTGGA	TGCGGCGTGC	GGTCGGCAAA	GACCAGACCG	TTCATACAGA	4320
ACTGGCGATC	GTTGSGCGTA	TGCCCAAAT	CACCGCCGTA	AGCCGACCAC	GGGTTGCCGT	4380
TTTCATCATA	TTTAATCAGC	GACTGATCCA	CCCAGTCCCA	GACGAAGCOG	CCCTGTAAAC	4440
GGGATACTG	ACGAAACGCC	TGCCAGTATT	TAGCGAAACC	GCCAAGACTG	TTACCCATOG	4500
CGTGGGCGTA	TTGCAAAGG	ATCAGCGGGC	GCGTCTCTCC	AGGTAGCGAA	AGCCATTTTT	4560
TGATGGACCA	TTTGGGCACA	GCOGGGAAGG	GCTGGTCTTC	ATCCACGCGC	GCGTACATCG	4620
GGCAAATAAT	ATCGGTGGCC	GTGGTGTCCG	CTCCGCGGCC	TTCATACTGC	ACOGGGCGGG	4680
AAGGATCGAC	AGATTTGATC	CAGCGATACA	GCGCGTCGTG	ATTAGCGCCG	TGGCCTGATT	4740
CATTCCCCAG	CGACCAGATG	ATCACACTCG	GGTGATTACG	ATCGCGCTGC	ACCATTGCGG	4800
TTACGCGTTC	GCTCATCGCC	GGTAGCCAGC	GCGGATCATC	GGTCAGACGA	TTGATTGGCA	4860
CCATGCGGTG	GGTTTCAATA	TTGGCTTCAT	CCACCACATA	CAGGCCGTAG	CGGTGCGACA	4920
GCGTGTACCA	CAGCGGATGG	TTGGGATAAT	GCGAACAGCG	CACGGCGTTA	AAGTTGTTCT	4980
GCTTCATCAG	CAGGATATCC	TGCACCATOG	TCGTCTCATC	CATGACCTGA	CCATGCAGAG	5040
GATGATGCTC	GTGACGGTTA	ACGCCTCGAA	TCAGCAAACGG	CTTGCCGTTC	AGCAGCAGCA	5100
GACCATTTTC	AATCOGCACC	TCGCGGAAAC	CGACATCGCA	GGCTTCTGCT	TCAATCAGCG	5160

This Sheet Has Been Replaced



TGCCGTCGGC	GGTGTGCAGT	TCAACCACCG	CACGATAGAG	ATTGGGGATT	TCGGCGCTCC	5220
ACAGTTTCGG	GTTTTTCGACC	TTGAGACGTA	GTGTGACGCG	ATCGGCATAA	CCACCACGCT	5280
CATCGATAAT	TTACACGCGG	AAAGGCGCGG	TGCGCTGGC	GACCTGCGTT	TCACCCTGCC	5340
ATAAAGAAAC	TGTTACCCGT	AGGTAGTCAC	GCAACTCGCC	GCACATCTGA	ACTTCAGCCT	5400
CCAGTACAGC	GCGGCTGAAA	TCATCATTAA	AGOGAGTGGC	AACATGGAAA	TCGCTGATTT	5460
GTGTAGTCGG	TTTATGCAGC	AACGAGACGT	CACGGAAAAT	GCCGCTCATC	CGCCACATAT	5520
CCTGATCTTC	CAGATAACTG	CCGTCACCTC	AAOCGAGCAC	CATCACCGCG	AGGCGGTTTT	5580
CTCCGGCGCG	TAAAAATGCG	CTCAGGTCAA	ATTCAGACGG	CAAACGACTG	TCCTGGCOGT	5640
AACCGACCCA	GCGCCCGTTG	CACCACAGAT	GAAACGCCGA	GTTAACGCCA	TCAAAAATAA	5700
TTGCGTCTG	GCCTTCCTGT	AGCCAGCTTT	CATCAACATT	AAATGTGAGC	GAGTAACAAC	5760
COGTGGATT	CTCCGTGGGA	ACAAACGGCG	GATTGACCGT	AATGGGATAG	GTTACGTTGG	5820
TGTAGATGGG	CGCATCGTAA	CCGTGCATCT	GCCAGTTTGA	GGGGACGACG	ACAGTATCGG	5880
CCTCAGGAAG	ATCGCACTCC	AGCCAGCTTT	COGGCACCGC	TTCTGGTGGC	GGAAACCAGG	5940
CAAAGCGCCA	TTCGCCATTC	AGGCTGCGCA	ACIGTTGGGA	AGGGCGATCG	GTGCGGGCCT	6000
CTTCGCTATT	ACGCCAGCTG	GCGAAAGGGG	GATGTGCTGC	AAGGOGATTA	AGTTGGGTAA	6060
CGCCAGGGTT	TTCCAGTCA	CGACGTTGTA	AAACGACGGG	ATCCCTCGAG	GAATTCATTT	6120
ATAGCATAGA	AAAAAACAAA	ATGAAATTCT	ACTATATTTT	TACATACATA	TATTCTAAT	6180
ATGAAAGTGG	TGATTGTGAC	TAGCGTAGCA	TCGCTTCTAG	ACATATACTA	TATAGTAATA	6240
CCAATACTCA	AGACTACGAA	ACTGATACAA	TCTCTTATCA	TGTGGGTAAT	GTTCTCGATG	6300
TCGAATAGCC	ATATGCCGGT	AGTTGCGATA	TACATAAACT	GATCACTAAT	TCCAAACCCA	6360
CCCGCTTTTT	ATAGTAAGTT	TTTCACCCAT	AAATAATAAA	TACAATAATT	AATTTCTCGT	6420
AAAAGTAGAA	AATATATTCT	AATTTATTGC	ACGGTAAGGA	AGTAGAATCA	TAAAGAACAG	6480
TGACGGATCC	CGGGATGGCC	ACACAGGGAC	AACGCGTCAA	CTGGGGAGAT	GAACCTTCCA	6540
AAAGACGTGG	TCGTTCTAAC	TCTCGTGGTC	GGAAGAATAA	TGATATACCT	TTGTCATTCT	6600
ACAACCCCAT	TACCCTCGAA	CAAGGATCTA	AATTTTGGA	TTTATGTCCG	AGAGACCTTG	6660
TTCCCAAAGG	AATAGGTAAT	AAGGATCAAC	AAATTGGTTA	TTGGAATAGA	CAGATTCGTT	6720
ATCGTATTGT	AAAAGGCCAG	CGTAAGGAAC	TCGCTGAGAG	GTGGTTCTTT	TACTTCITAG	6780

This Sheet Has Been Replaced



GTACAGGACC TCATGCTGAT GCTAAATTCA AAGACAAGAT TGATGGAGTC TTCTGGGTTG	6840
CAAGGGATGG TGCCATGAAC AAGCCCACAA CGCTTGGCAC TCGTGGAACC AATAACGAAT	6900
CCAAACCACT GAGATTTGAT GGTAAGATAC CGCCACAGTT TCAGCTTGAA GTGAACCGTT	6960
CTAGGAACAA TTCAAGGTCT GGTTCCTCAGT CTAGATCTGT TTCAAGAAAC AGATCTCAAT	7020
CTAGAGGAAG ACACCATTCC AATAACCAGA ATAATAATGT TGAGGATACA ATTGTAGCCG	7080
TGCTTGAAAA ATTAGGTGTT ACTGACAAAC AAAGGTCACG TTCTAAACCT AGAGAACGTA	7140
GTGATTCCAA ACCTAGGGAC ACAACACCTA AGAATGCCAA CAAACACACC TGAAGAAAA	7200
CTGCAGGCAA GGGAGATGTG ACAACTTTCT ATGGTGCTAG AAGTAGTTCA GCTAACTTTG	7260
GTGATAGTGA TCTCGTTGCC AATGGTAAAG CTGCCAAATG CTACCCTCAG ATAGCTGAAT	7320
GTGTTCCATC AGTGTCTAGC ATAATCTTTG GCAGTCAATG GTCGTCTGAA GAAGCTGGTG	7380
ATCAAGTGAA AGTCACGCTC ACTCACACCT ACTACCTGCC AAAGGATGAT GCCAAACTA	7440
GTCAATTCCCT AGAACAGATT GACGCTTACA AGCGACCTTC TGAAGTGGCT AAGGATCAGA	7500
GGCAAAGAAG ATCCCGTTCT AAGTCTGCTG ATAAGAAGCC TGAGGAGTTG TCTGTAACTC	7560
TTGTGGAGGC ATACACAGAT GTGTTTGATG ACACACAGGT TGAGATGATT GATGAGGTTA	7620
CGAACTAAAC GCATGCCCGG GAATTCTGTG AGCGTATGGC AAACGAAGGA AAAATTAGTT	7680
ATAGTAGCCG CACTCGATGG GACATTTCAA CGTAAACCGT TTAATAATAT TTTGAATCTT	7740
ATTCCATTAT CTGAAATGGT GGTAAACTA ACTGCTGTGT GTATGAAATG CTTTAAGGAG	7800
GCTTCCTTTT CTAAAGATT GGGTGAGGAA ACCGAGATAG AAATAATAGG AGGTAATGAT	7860
ATGIATCAAT CGGTGTGTAG AAAGTGTTAC ATCGACTCAT AATATTATAT TTTTATCTA	7920
AAAACTAAA AATAACATT GATTAAATTT TAATATAATA CTTAAAATG GATGTTGTGT	7980
CGTTAGATAA ACCGTTTATG TATTTTGAGG AAATTGATAA TGAGTTAGAT TACGAACCAG	8040
AAAGTGCAAA TGAGGTGCA AAAAACTGC CGTATCAAGG ACAGTTAAAA CTATTACTAG	8100
GAGAATTATT TTTCTTAGT AAGTTACAGC GACACGGTAT ATTAGATGGT GCCACCGTAG	8160
TGTATATAGG ATCTGCTCCC GGTACACATA TACGTTATTT GAGAGATCAT TTCTATAATT	8220
TAGGAGTGAT CATCAAATGG ATGCTAATTG ACGGCCGCCA TCATGATCCT ATTTTAAATG	8280
GATTGCGTGA TGTGACTCTA GTGACTCGGT TCGTTGATGA GGAATATCTA CGATCCATCA	8340
AAAAACAAC GCATCCTTCT AAGATTATTT TAATTTCTGA TGTGAGATCC AAACGAGGAG	8400

This Sheet Has Been Replaced



GAAATGAACC TAGTAOGGCG GATTTACTAA GTAATTACGC TCTACAAAAT GTCATGATTA	8460
GTATTTTAAA CCCOGTGGCG TCTAGTCTTA AATGGAGATG CCOGTTTCCA GATCAATGGA	8520
TCAAGGACTT TTATATCCCA CACGGTAATA AAATGTTACA ACCTTTTGCT CCTTCATATT	8580
CAGGGCCGTC GTTTTACAAC GTOGTGACTG GGAAAACCCT GCGGTTACCC AACTTAATCG	8640
CCTTGCAGCA CATCCCCCTT TCGCCAGCTG GCGTAATAGC GAAGAGGCCG GCACOGATOG	8700
CCCTTCCCAA CAGTTGCGCA GCCTGAATGG CGAATGGGCG CTGATGCGGT ATTTTCTCTT	8760
TACGCATCTG TCGGGTATTT CACACOGCAT ATGGTGCACT CTCAGTACCA TCTGCTCTGA	8820
TGCOGCATAG TTAAGCCAGT ACACTCOGCT ATOGCTACGT GACTGGGTCA TGGCTGCGCC	8880
COGACACCCG CCAACACCCG CTGACGGGCG CTGACGGGCT TGTCTGCTCC OGGCATCOGC	8940
TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC	9000
ACOGAAACGC GCGAGGCAG	9019

This Sheet Has Been Replaced



What is claimed is:

1. A recombinant raccoon poxvirus having at least one internal gene comprising a DNA sequence encoding a member selected from the group consisting of the nucleocapsid (N) and transmembrane (M/E1) proteins of Feline Infectious Peritonitis Virus (FIPV).
2. The recombinant raccoon poxvirus of claim 1 wherein said internal gene encodes the N protein of FIPV having the amino acid sequence as set out in Figure 1B.
3. The recombinant raccoon poxvirus of claim 1 wherein said internal gene encodes the M/E1 protein of FIPV having the amino acid sequence as set out in Figure 1A.
4. The recombinant raccoon poxvirus of claim 1 wherein said virus has genes encoding the E1 and N proteins of FIPV.
5. A vaccine comprising:
a recombinant raccoon poxvirus having at least one internal gene comprising a DNA sequence encoding a member selected from the group consisting of the nucleocapsid (N) and transmembrane (M/E1) proteins of Feline Infectious Peritonitis Virus (FIPV),
a pharmaceutically acceptable carrier or diluent, and
a pharmaceutically acceptable adjuvant.
6. The vaccine of claim 5 wherein said internal gene encodes the N protein of FIPV having the amino acid sequence as set out in Figure 1B.
7. The vaccine of claim 5 wherein said internal gene encodes the

This Sheet Has Been Replaced



M/E1 protein of FIPV having the amino acid sequence as set out in Figure 1A.

8. The vaccine of claim 5 wherein said virus has genes encoding the E1 and N proteins of FIPV.

9. The vaccine of claim 5 further comprising inactivated or attenuated viruses selected from the group consisting of feline leukemia virus, feline panleucopenia virus, feline rhinotracheitis virus, feline calicivirus, feline immunodeficiency virus, feline herpesvirus, feline enteric coronavirus, or mixtures thereof.

10. The vaccine of claim 5 further comprising inactivated or attenuated feline *Chlamydia psittaci*, *Microsporium canis*, or mixtures thereof.

11. A vaccine comprising:

a first recombinant raccoon poxvirus having at least one internal gene comprising a DNA sequence encoding a member selected from the group consisting of the nucleocapsid (N) and transmembrane (M/E1) proteins of Feline Infectious Peritonitis Virus (FIPV),

a second recombinant raccoon poxvirus having at least one internal gene comprising a DNA sequence encoding a member selected from the group consisting of the nucleocapsid (N) and transmembrane (M/E1) proteins of Feline Infectious Peritonitis Virus (FIPV),

a pharmaceutically acceptable carrier or diluent, and

a pharmaceutically acceptable adjuvant.

12. A method for preventing disease caused by Feline Infectious Peritonitis Virus (FIPV), comprising administering to a feline in need of such treatment a vaccine comprising a recombinant raccoon poxvirus having at least one internal gene comprising a DNA sequence encoding a member selected from the group consisting of





AHP-93116

-35-

the nucleocapsid (N) and transmembrane (M/E1) proteins of FIPV.

13. The method of claim 12 wherein said internal gene encodes the N protein of FIPV having the amino acid sequence as set out in Figure 1B.

14. The method of claim 12 wherein said internal gene encodes the M/E1 protein of FIPV having the amino acid sequence as set out in Figure 1A.

15. The method of claim 12 wherein said virus has genes encoding the E1 and N proteins of FIPV.

This Sheet Has Been Replaced

