

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

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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.

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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.

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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

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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 recombina-
5 recombinatorial 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
10 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
20 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
25 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

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

5 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.

15 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.

20 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.

25 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

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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

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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.

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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

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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
15 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.

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

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(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



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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 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

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 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 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

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

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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

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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

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(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).

Example 2

PREPARATION OF VACCINE AND TESTING FOR EFFICACY IN CATS

1. Preparation of Master Seeds of RRPV-FIPV N and El 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

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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

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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:

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<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.	
10	Dehydration	1 point/day
	Depression	1 point/day
	Anorexia	1 point/day
	Peritoneal Swelling	1 point/day
	Icterus	1 point/day
	Weight Loss	>20% 1 point per observation
15		>30% 2 points per observation
		>50% 5 points per observation
	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

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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.

5

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.

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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	6
1305	0	1	0	0	6	0	6
1307	2	1	0	0	4	25	33
1309	2	1	6	0	6	25	40
1311	4	3	0	0	18	25	50
1/10 DOSE SUBCUTANEOUS VACCINATES							
1313	29	5	9	3	36	35	107
1315	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	19	3	63	25	161
1323	5	0	2	0	14	25	46
1327	0	1	2	0	15	25	43
1329	6	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 TATTGGCAT CTGCTAACT GGAACCTCAG CTGGTCTGTA	180
ATATTGATTG TTTTATAAC AGTGTTACAA TATGGCAGAC CACAATTAG CTGGCTCGTT	240
TATGGCATTA AAATGCTGAT CATGTGGCTA TTATGGCCTA TTGTTCTAGC GCTTACGATT	300
TTTAATGCAT ACTCTGAGTA CCAAGTTTCC AGATATGTAA TGTTOGGCTT TAGTGTTGCA	360
GGTGCAATTG TAACGTTTGC ACTTTGGATG ATGTATTTTG TGAGATCTGT TCAGCTATAT	420
AGAAGAACCA AATCATGGTG GTCTTTTAAT CCTGAGACTA ATGCAATTCT TTGTGTTAAT	480
GCATTGGGTA GAAGTTATGT GCTTCCCTTA GATGGTACTC CTACAGGTGT TACCCCTACT	540
CTACTTTCAG GAAATCTATA TGCTGAAGGT TTCAAATGG CTGGTGGTTT AACCATOGAG	600
CATTTCCTA AATACGTCAT GATGCTACA CCTAGTAGAA CCATCGTTTA TACATPAGTT	660
GGAAAACAAT TAAAAGCAAC TACTGCCACA GGATGGGCTT ACTACGTAAA 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 TGGCACTGGT GGAACCAATA ACGAATCCAA ACCACTGAGA	420
TTTGATGGTA AGATACCGCC ACAGTTTCAG CTTGAAGTGA ACCGTTCTAG GAACAATTCA	480
AGGTCIGGTT CTCAGTCTAG ATCTGTTTCA AGAAACAGAT CTCAATCTAG AGGAAGACAC	540
CATTCCAATA ACCAGAATAA TAATGTTGAG GATACAATTG TAGCOGTGCT TGAAAAATTA	600
GGTGTACTG ACAAACAAAG GTCAGTTTCT AAACCTAGAG AACGTAGTGA TTCCAAACCT	660
AGGGACACAA CACCTAAGAA TGCCAACAAA CACACCTGGA AGAAAACGTC AGGCAAGGGA	720
GATGTGACAA CTTTCTATGG TGCTAGAAGT AGTTCAGCTA ACTTTGGTGA TAGTGATCTC	780
GTGCCAATG GTAAAGCTGC CAAATGCTAC CCTCAGATAG CTGAATGTGT TCCATCAGTG	840
TCTAGCATAA TCTTTGGCAG TCAATGGTCT GCTGAAGAAG CTGGTGATCA AGTGAAAGTC	900
ACGCTCACTC ACACCTACTA CCTGCCAAAG GATGATGCCA AAACCTAGTCA ATTCCTAGAA	960
CAGATTGACG CTTACAAGCG ACCTTCTGAA GTGGCTAAGG ATCAGAGGCA AAGAAGATCC	1020
CGTTCPAAGT 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 CCTATTITTA TAGGTTAATG TCATGATAAT AATGGTTTCT	60
TAGACGTCAG GTGGCACATT TOGGGGGAAT GGGGCGGAA CCCCTATTG TTTATTITTC	120
TAAATACATT CAAATATGTA TCOGCTCATG AGACAATAAC CCTGATAAAT GCTTCAATTA	180
TATTGAAAAA GGAAGAGTAT GAGTATTCAA CATTTCCGTG TOGCCCTTAT TCCCTITTTT	240
GCGGCATTTT GCCTTCCTGT TTTGCTCAC CCAGAAAGCG TGGTGAAGT AAAAGATGCT	300
GAAGATCAGT TGGGTGCAAG AGTGGGTAC ATGAACTGG ATCTCAGAG CGGTAAGATC	360
CTGAGAGTT TCGCCCCGA AGAAGCTTTT CCAATGATGA GCACTTTTAA AGTTCTGCTA	420
TGTTGGCGGG TATTATCCCG TATTGAAGCC GGGCAAGAGC AACTCGGTG CCGCATACAC	480
TATCTCAGA ATGACTTGGT TGAGTACTCA CCACTCAGAG AAAAGCATCT TACGGATGGC	540
ATGACAGTAA GAGAATTATG CAGTCTGCC ATAACATGA GTGATACAC TCGGGCCAAC	600
TTACTTCGA CAAAGATCGG AGGACCGAAG GAGCTAACCG CTTTTTGCA CACATGGGG	660
GATCATGTAA CTGGCTTGA TCGTTGGGAA CCGGAGCTGA ATGAAGCCAT ACCAAACGAC	720
GAGCGTGACA CCAGGTGCC TGTAGCAATG GCAACAAGT TCGCAAACT ATTAAGTGGC	780
GAAGTACTTA CTCTAGCTTC CCGCAACAA TTAATAGACT GGATGGAGGC GGATAAGTT	840
GCAGGACCAC TTCAGCGCTC GGCCCTTCOG GCTGGCTGGT TTATTGCTGA TAAATCTGGA	900
GCGGTGAGC GTGGGTCTOG CGGTATCATT GCAGCACTGG GGCAGATGG TAAGCCCTCC	960
CGTATCGTAG TTATCTACAC GACGGGGAGT CAGGCAACTA TGGATGAACG AAATAGACAG	1020
ATCGCTGAGA TAGGTGCCCTC ACTGATTAAAG CATTTGTAAC TGTGAGACCA AGTTTACTCA	1080
TATATACTTT AGATTGATTT ARAACTTCAT TTTAATTTA AAAGGATCTA GGTGAAGATC	1140
CTTTTGTATA ATCTCATGAC CAAAATCCCT TACGTGAGT TTGGTTCCA CTGAGCGTCA	1200
GACCCGTTAG AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC	1260
TGCTTGCAAA CAAAAAACC ACGCTACCA GCGGTGGTTT GTTTGCCGA TCAGAGCTA	1320
CCAACTCTTT TTCCGAGGT AACTGGCTTC AGCAGAGCGC AGATACCABA TACTGTCTT	1380
CTAGTGTAGC CGTAGTTAGG CCACCACTTC AAGAAGCTG TAGCACCGCC TACATACCTC	1440
GCTCTCTAA TCCGTATACC AGTGGCTGCT GCCAGTGGG ATAAGTCGTG TCTTACCGGG	1500

This Sheet Has Been Replaced



TTGGACTCAA	GACGATAGTT	ACCGGATAAG	GCGCAGCGGT	CGGGCTGAAC	GGGGGGTTGG	1560
TGCACACAGC	CCAGCTTGGA	GOGAAOGACC	TACACOGAAC	TGAGATACCT	ACAGCGTGAG	1620
CATTGAGAAA	GOGCCACGCT	TCCCGAAGGG	AGAAAGGOGG	ACAGGTATCC	GGTAAGCGGC	1680
AGGGTCGGAA	CAGGAGAGCG	CACGAGGGAG	CTTCCAGGGG	GAAACGGCTG	GTATCTTTAT	1740
AGTCCGTGTC	GGTTTCGCCA	CCTCTGACTT	GAGCGTCGAT	TTTTGTGATG	CTGCTCAGGG	1800
GGGCGGAGCC	TATGGAAAAA	OGOCAGCAAC	GOGGCCTTTT	TACGGTTCTT	GGCCTTTTGC	1860
TGGCCTTTTG	CTCACATGTT	CTTTCCTGCG	TTATCCCCTG	ATTCTGTGGA	TAACCGTATT	1920
ACCGCCTTTG	AGTGAGCTGA	TACCGCTCGC	CGCAGCGGAA	CGACCGAGCG	CAGCGAGTCA	1980
GTGAGCGAGG	AAGCGGAAGA	GCGCCCAATA	CGCAAACCGC	CTCTCCCGCG	GCGTTGGCCG	2040
ATTCATTAAAT	GCGGCTGGCA	CGACAGGTTT	CCCGACIGGA	AAGCGGGCAG	TGAGCGCAAC	2100
GCAATTAATG	TGAGTTAGCT	CACTCATTAG	GCACCCGAGG	CTTACACTTT	TATGCTTCGG	2160
GCTCGTATGT	TGTGTGGAAT	TGTGAGOGGA	TAACAATTTC	ACACAGSAAA	CAGCTATGAC	2220
CATGATTACG	CCAAGCTTTT	GCGATCAATA	AATGGATCAC	AACCAGTATC	TCTTAAOGAT	2280
GTTCTTCGCA	GATGATGATT	CATTTTTTAA	GTATTIGGCT	AGTCAAGATG	ATGAAATCTT	2340
CATTATCTGA	TATATTCGAA	ATCACTCAAT	ATCTAGACTT	TCTGTATTTA	TTATTGATCC	2400
AATCAAAAAA	TAAATTAGAA	GCCGTGGGTC	ATTGTTATGA	ATCTCTTTCA	GAGGAATACA	2460
GACAATTGAC	AAAATTCACA	GACTTTCAAG	ATTTTAAAAA	ACTGTTTAAAC	AAGGTCCCTA	2520
TTGTTACAGA	TGGAAGGGTC	AAACTTAATA	AAGGATATTT	GTTCGACTTT	GTGATTAGTT	2580
TCATGCGATT	CAAAAAGGAA	TCCTCTCTAG	CTACCACCGC	AATAGATCCT	GTTAGATACA	2640
TAGATCCTCG	TCGCAATATC	GCATTTTCTA	ACGTGATGGA	TATATTAAAG	TCGAATAAAG	2700
TGAACAATAA	TTAATTCTTT	ATTGTCATCA	TGAACGGCGG	ACATATTCAG	TIGATAATCG	2760
GCCCCATGTT	TTCAGGTAAA	AGTACAGAAT	TAATTAGACG	AGTTAGACGT	TATCAAATAG	2820
CTCAATATAA	ATGCGTGACT	ATAAAATATT	CTAACGATAA	TAGATACGGA	ACGGGACTAT	2880
GGACGCATGA	TAAGAATAAT	TTTGAGGCAT	TGGAAGCAAC	TAAACTATGT	GATCTCTTGG	2940
AATCAATTAC	AGATTTCTCC	GIGATAGGTA	TCGATGAAGG	ACAGTTCTTT	CCAGACATTG	3000
TIGAATTCCG	AGCTTGGCTG	CAGGTGGGGG	ATCCCCCCTG	CCCGGTTATT	ATTATTTTTG	3060
ACACCAGACC	AACTGGTAAT	GGTAGOGAAC	GGCGCTCAGC	TGAATTCCGC	CGATACTGAC	3120

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GGGCTCCAGG	AGTCGTCCGC	ACCAATCCCC	ATATGGAAAC	CGTCGATATT	CAGCCATGTG	3180
CCTTCTTCOG	CGTGCAGCAG	ATGGCGATGG	CTGGTTTCCA	TCAGTTGCTG	TTGACTGTAG	3240
CGGCTGATGT	TGAACTGGAA	GTCGCCGCGC	CACTGGTGTG	GGCCATAATT	CAATTCGCGC	3300
GTCCCGCAGC	GCAGACCGTT	TTGCTCGGG	AAGACGTACG	GGGTATACAT	GTCTGACAAT	3360
GGCAGATCCC	AGCGGTCAAA	ACAGGCGGCA	GTAAGCGGGT	CGGGATAGTT	TTCTTGCGGC	3420
CCTAATCCGA	GCCAGTTTAC	CGCTCTGCT	ACCTGCGCCA	GCTGGCAGTT	CAGGCCAATC	3480
CGCGCCGGAT	GCGGTGTATC	GCTCGCCACT	TCAACATCAA	CGGTAATCGC	CATTTGACCA	3540
CTACCATCAA	TCCGGTAGGT	TTTCCGGCTG	ATAAATAAGG	TTTCCCCCTG	ATGCTGCCAC	3600
GCGTGACCGG	TGTAATCAG	CACCGCATCA	GCAAGTGTAT	CTGCCGTGCA	CTGCAACAAC	3660
GCTGCTTCGG	CCTGGTAATG	GCCCGCCGCC	TTCCAGCGTT	CGACCCAGGC	GTTAGGGTCA	3720
ATGCGGGTCG	CTTCACTTAC	GCCAATGTGG	TTATCCAGCG	GTGCACGGGT	GAACGTATCG	3780
CGCAGCGGCG	TCAGCAGTTG	TTTTTTATCG	CCAATCCACA	TCGTGAAAG	AAAGCCTGAC	3840
TGGCGGTTAA	ATTGCCAACC	CTTATTACCC	AGCTCGATGC	AAAAATCCAT	TTGCTGGTGG	3900
GTCAGATGCG	GGATGGCGTG	GGACGCGGCG	GGGAGCGTCA	CACTGAGGTT	TTCCGCCAGA	3960
CGCCACTGCT	GCCAGGCGCT	GATGTGCCCG	GCTTCTGACC	ATGCGGTGCG	GTTCCGGTTGC	4020
ACTACGGGTA	CTGTGAGCCA	GAGTTGCCCG	GCGCTCTCCG	GCTGCGGTAG	TTCAGGCAGT	4080
TCAATCAACT	GTTTACCTTG	TGGAGCGACA	TCCAGAGGCA	CTTCACCGCT	TGCCAGCGGC	4140
TTACCATCCA	GCGCCACCAT	CCAGTGCGAG	AGCTCGTTAT	CGCTATGACG	GAACAGGTAT	4200
TCGCTGGTCA	CTTCGATGGT	TTGCCCGGAT	AAACGGAACT	GGAAAAACTG	CTGCTGGTGT	4260
TTTGCTTCCG	TCAGCGCTGG	ATGCGGCGTG	CGGTCCGCAA	AGACCAGACC	GTTCATAACG	4320
AACTGGCGAT	CGTTCCGGGT	ATCGCCAAAA	TCACCGCGGT	AAGCCGACCA	CGGGTTGCCG	4380
TTTTCATCAT	ATTTAATCAG	CGACTGATCC	ACCCAGTCCC	AGACGAAGCC	GCCCCTGTAA	4440
CGGGGATACT	GACGAAACGC	CTGCCAGTAT	TTAGCGAAAC	CGCCAAGACT	GTTACCCATC	4500
GCGTGGGCGT	ATTGCAAAG	GATCAGCGGG	CGGTCCTCTC	CAGGTAGCGA	AAGCCATTTT	4560
TTGATGGACC	ATTTCCGCAC	AGCCGGGAAG	GGCTGGTCTT	CATCCACGCG	CGCGTACATC	4620
GGGCAAATAA	TATCGGTGGC	CGTGGTGTGG	GCTCCGCCGC	CTTCATACTG	CACCGGGGCG	4680
GAAGGATCGA	CAGATTGAT	CCAGCGATAC	AGCGCGTCGT	GATTAGCGCC	GTGGCCTGAT	4740

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TCATTCCCCA	GCGACCAGAT	GATCACACTC	GGGTGATTAC	GATCGCGCTG	CACCATTCGC	4800
GTACGCGTT	CGCTCATCGC	CGGTAGCCAG	CGCGGATCAT	CGGTCAGACG	ATTGATTGGC	4860
ACCATGCCGT	GGGTTTCAAT	ATTGGCTTCA	TCCACCACAT	ACAGGCGGTA	GCGGTGCGAC	4920
AGCGTGACC	ACAGCGGATG	GTTCGGATAA	TGCGAACAGC	GCACGGCGTT	AAAGTTGTTT	4980
TGCTTCATCA	GCAGGATATC	CTGCACCATC	GTCCTGCTCAT	CCATGACCTG	ACCATGCAGA	5040
GGATGATGCT	CGTGACGGTT	AAAGCCTCGA	ATCAGCAACG	GCTTGCGGTT	CAGCAGCAGC	5100
AGACCATTTT	CAATCCGCB	CTCGCGGAAA	CGGACATCGC	AGGCTTCTGC	TTCAATCAGC	5160
GTGCGGTGG	CGGTGTGCAG	TTCAACCACC	GCACGATAGA	GATTGGGGAT	TTCGGCGCTC	5220
CACAGTTTCG	GGTTTTCGAC	CTTGAGAGCT	AGTGTGACGC	GATCGGCATA	ACCACCACGC	5280
TCATCGATAA	TTTCACCGCC	GAAAGGCGCG	GTGCGCGCTG	CGACCTGGGT	TTACCCCTGC	5340
CATAAAGAAA	CTGTTACCCG	TAGGTAGTCA	CGCAACTCGC	CGCACATCTG	AACTTCAGCC	5400
TCCAGTACAG	CGCGGCTGAA	ATCATCATT	AAGCGAGTGG	CAACATGGAA	ATCGCTGATT	5460
TGTTAGTTCG	GTTTATGCAG	CAACGAGACG	TCAAGGAAA	TGCGGCTCAT	CGCCACATA	5520
TCTGATCTT	CCAGATAACT	GCGTCACTC	CAACGCAGCA	CCATCACCGC	GAGGCGGTTT	5580
TCTCGCGCG	GTAAAAATGC	GCTCAGGTCA	AATTCAGACG	GCBAAGACT	GTCCCGGCGG	5640
TAACCGACCC	AGCGCCCGTT	GCACCACAGA	TGAAACGCGG	AGTTAAGGCC	ATCAAAATA	5700
ATTCGGGTCT	GGCCTTCCTG	TAGCCAGCTT	TCATCAACAT	TAAATGTGAG	CGAGTAACAA	5760
CCCGTGGGAT	TCTCCGTGGG	AACAAACGGC	GGATTGACCG	TAATGGGATA	GGTTACGTTG	5820
GTTAGATGG	GCGCATCGTA	ACCGTGCATC	TGCCAGTTTG	AGGGGACGAC	GACAGTATCG	5880
GCCTCAGGAA	GATCGCACTC	CAGOCAGCTT	TCCGGCACCG	CTTCTGGTGC	CGAAACCCG	5940
GCAAAGCGCC	ATTCGCCATT	CAGGCTGCGC	AACGTGTTGG	AAGGGCGATC	GGTGGCGGCC	6000
TCTTCGCTAT	TACGCCAGCT	GGCGBAAGGG	GGATGTGCTG	CAAGGCGATT	AAGTTGGGTA	6060
ACGCCAGGGT	TTCCCGAGTC	ACGACGTTGT	AAAACGACGG	GATCCCTCGA	GGAATTCATT	6120
TATAGCATAG	AAAAAAACAA	AATGAAATTC	TACTATATTT	TTACATACAT	ATATTCTAAA	6180
TATGAAAGTG	GTGATTGTGA	CTAGCGTAGC	ATCGCTTCTA	GACATATACT	ATATAGTAAT	6240
ACCAATACTC	AAGACTACGA	AACTGATACA	ATCTCTTATC	ATGTGGGTAA	TGTTCTCGAT	6300
GTCGAATAGC	CATATGCCGG	TAGTTGCGAT	ATACATAAAC	TGATCACTAA	TTCCAAACCC	6360

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ACCCGCTTTT	TATAGTAAAGT	TTTTCACCCA	TAAATAATAA	ATACAATAAT	TAATTTCTCG	6420
TAAAAGTAGA	AAATATATTC	TAATTTATTG	CACGGTAAGG	AAGTAGAATC	ATAAAGAACA	6480
GTGAOGGATC	CCAATTOGGG	CATTTTGGT	TTGAACTAAA	CAAAATGAAG	TACATTTTGC	6540
TAATACTOGC	GTGCATAATT	GCATGCGTTT	ATGGTGAACG	CTACTGTGCC	ATGCAAGACA	6600
GTGGCTTGCA	GTGTATTAAT	GGCACAAATT	CAAGATGTCA	AACCTGCTTT	GAACGTGGTG	6660
ATCTTATTTG	GCATCTTGCT	AACTGGAACT	TCAGCTGGTC	TGTAATATTG	ATTGTTTTTA	6720
TAACAGTGTT	ACAATATGGC	AGACCACAAT	TTAGCTGGCT	OGTTTATGGC	ATTAAAATGC	6780
TGATCATGTG	GCTATTATGG	CCTATTGTTT	TAGCGCTTAC	GATTTTTAAT	GCATACTCTG	6840
AGTACCAAGT	TTCCAGATAT	GTAATGTTGG	GCITTAGTGT	TGCAGGTGCA	GTGTGAACGT	6900
TTGCACTTTG	GATGATGTAT	TTGTGAGAT	CTGTTTCACT	ATATAGAAGA	ACCAAATCAT	6960
GGTGGTCTTT	TAATCCTGAG	ACTAATGCAA	TTCTTTGTTT	TAATGCATTG	GGTAGAAGTT	7020
ATGTGCTTCC	CTTAGATGGT	ACTCCTACAG	GTGTTACCTT	TACTCTACTT	TCAGGAAATC	7080
TATATGCTGA	AGGTTTCAAA	ATGGCTGGTG	GTTTAACCAT	CGAGCATTTG	CCTAAATACG	7140
TCATGATTGC	TACACCTAGT	AGAACCATCG	TTTATACATT	AGTTGGAAAA	CAATTAAAAG	7200
CAACTACTGC	CACAGGATGG	GCTTACTACG	TAAATCTTAA	AGCTGGTGAT	TACTCAACAG	7260
AAGCAOGTAC	TGACAATTTG	AGTGAACATG	AAAAATTATT	ACATATGGTG	TAACTAAACT	7320
TTCAAATGGG	GGAATTCCTG	GAGCGTATCG	CAAAOGAAGG	AAAAATTAGT	TATAGTAGCC	7380
GCACTCGATG	GGACATTTCA	ACGTAAACCG	TTTAATAATA	TTTTGAATCT	TATTCATTAA	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

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ATGTGACTCT AGTGACTCGG TTCGTTGATG AGGAATATCT ACGATCCATC AAAAAACAAC	8040
TGCATCCTTC TAAGATTATT TTAATTTCTG ATGTGAGATC CAAACGAGGA GGAAATGAAC	8100
CTAGTACGGC GGATTFACTA AGTAATTACG CTCTACAAA TGTCATGATT AGTATTTTAA	8160
ACCCCGTGGC GTCTAGTCTT AAATGGAGAT GCCCGTTTCC AGATCAATGG ATCAAGGACT	8220
TTTATATCCC ACACGGTAAT AAAATGTTAC AACCTTTTGC TCCTTCATAT TCAGGGCCGT	8280
CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC	8340
ACATCCCCCT TTGCCAGCT GCGTAATAG CGAAGAGGCC CGCACCAGTC GCCCTTCCCA	8400
ACAGTTGCGC AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCT TTACGCATCT	8460
GTGCGGTATT TCACACGCA TATGGTGCAC TCTCAGTACC ATCTGCTCTG ATGCGGCATA	8520
GTTAAGCCAG TACACTCCGC TATCGCTACG TGACTGGGTC ATGGCTGCGC CCGACACCC	8580
GCCAACACCC GCTGACGCGC CCTGACGGGC TTGTCGTCTC CGGCATCCG CTTACAGACA	8640
AGCTGTGACC GTCTCCGGGA GCTGCATGTG 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 CTATTTTTAT AGGTTAATGT CATGATAATA ATGGTTTCCT	60
AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCGTATTGT TTATTTTTCT	120
AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT	180
ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT CCGTTTTTGT	240
CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG	300

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AAGATCAGTT	GGGTGCACGA	GTGGGTACAA	TGGAAGTGG	TCTCAACAGC	GGTAAGATCC	360
TTGAGAGTTT	TCGCCCCGAA	GAACGTTTTT	CAATGATGAG	CACTTTTTAA	GTTCTGCTAT	420
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ATTCTCAGAA	TGACTTGGTT	GAGTACTCAC	CAGTCACAGA	AAAGCATCTT	ACGGATGGCA	540
TGACAGTAAG	AGAATTATGC	AGTGCTGCCA	TAACCATGAG	TGATAACACT	GCGGCCAACT	600
TACTTCTGAC	AACGATCGGA	GGACCGAAGG	AGCTAACCGC	TTTTTTGCAC	AACATGGGGG	660
ATCATGTAAC	TCGCCTTGAT	CGTTGGGAAC	CGGAGCTGAA	TGAAGCCATA	CCAAACGACG	720
AGCGTGACAC	CACGATGCCT	GTAGCAATGG	CAACAACGTT	GCGCAAACCT	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	CTGATTAAGC	ATTGGTAACT	GTCAGACCAA	GTTTACTCAT	1080
ATATACTTTA	GATTGATTTA	AAACTTCATT	TTTAATTTAA	AAGGATCTAG	GTGAAGATCC	1140
TTTTTGATAA	TCTCATGACC	AAAATCCCTT	AACGTGAGTT	TTCGTTCCAC	TGAGCGTCAG	1200
ACCCCGTAGA	AAAGATCAAA	GGATCTTCTT	GAGATCCCTT	TTTTCTGCGC	GTAATCTGCT	1260
GCTTGCAAAC	AAAAAAACCA	CGCTACCAG	CGGTGGTTTG	TTTGCCGGAT	CAAGAGCTAC	1320
CAACTCTTTT	TCGAAGGTA	ACTGGCTTCA	GCAGAGCGCA	GATACCAAAT	ACTGTCCCTC	1380
TAGTGTAGCC	GTAGTTAGGC	CACCACITCA	AGAACTCTGT	AGCACCGCCT	ACATACCTCG	1440
CTCTGCTAAT	CCTGTTACCA	GTGGCTGCTG	CCAGTGGCGA	TAAGTCGTGT	CTTACCGGGT	1500
TGGAATCAAG	ACGATAGTTA	COGATAAGG	CGCAGCGGTC	GGGCTGAACG	GGGGGTTCGT	1560
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ATTGAGAAAG	CGCCACGCTT	CCCGAAGGGA	GAAAGGCGGA	CAGGTATCCG	GTAAGCGGCA	1680
GGGTGGAAC	AGGAGAGCGC	ACGAGGGAGC	TTCCAGGGGG	AAACGCCTGG	TATCTTTATA	1740
GTCCGTGTCG	GTTTCGCCAC	CTCTGACTTG	AGCGTCGATT	TTGTGATGTC	TCGTCAGGGG	1800
GGCGGAGCCT	ATGGAAAAC	GCCAGCAACG	CGGCTTTTTT	ACGGTTCCTG	GCCTTTTGCT	1860
GGCCTTTTGC	TCACATGTTT	TTTCTGCGT	TATCCCTTGA	TTCTGTGGAT	AACCGTATTA	1920

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COGCCCTTTGA GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACOGAGCGC AGOGAGTCAG	1980
TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG CGTTGGCOGA	2040
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CAATTAATGT GAGTTAGCTC ACTCATTAGG CACCCCAGGC TTTACACTTT ATGCTTCOGG	2160
CTOGTATGTT GTGTGGAATT GTGAGCGGAT AACAAATTTCA CACAGGAAAC AGCTATGACC	2220
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TTCTTCGCAG ATGATGATTC ATTTTPTAAG TATTTGGCTA GTCAAGATGA TGAAATCTTC	2340
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TCAATATAAA TGCGTGACTA TAAAATATTC TAACGATAAT AGATACGGAA CGGGACTATG	2880
GACGCATGAT AAGAATAATT TTGAAGCATT GGAAGCAACT AACTATGTG ATCTCTTGGA	2940
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CTTCTTCOGC GTGCAGCAGA TGGCGATGGC TGGTTTCCAT CAGTTGCTGT TGACTGTAGC	3240
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GCGCOGGATG CSGTGTATCG CTCGCCACTT CAACATCAAC GGTAAATCGCC ATTTGACCAC	3540

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AAAGTGCAAA	TGAGGTGCGA	AAAAAACTGC	CGTATCAAGG	ACAGTTAAAA	CTATTACTAG	8100
GAGAATTATT	TTTTCTTAGT	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

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GAAATGAACC TAGTAOGGCG GATTFACTAA GTAATTACGC TCTACAAAAT GTCATGATTA	8460
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CCTTGCAGCA CATCCCCCTT TOGCCAGCTG GCGTAATAGC GAAGAGGCC GCACCGATCG	8700
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TACGCATCTG TCGGTTATTT CACACOGCAT ATGGTGCACT CTCAGTACCA TCTGCTCTGA	8820
TGCOGCATAG TTAAGCCAGT AACTTCOGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC	8880
COGACACCCG CCAACACCCG CTGAOGGGCC CTGAOGGGCT TGTCTGCTCC CGGCATCCGC	8940
TTACAGACAA GCTGTGACCG TCTCOGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC	9000
ACOGAAACGC GCGAGGCAG	9019

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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





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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

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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.

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

The invention that relates to recombinant raccoon poxvirus useful as a Vaccine Against Feline Infectious Peritonitis Virus Disease. The recombinant raccoon poxvirus has 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).

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Sequence Range: 1 to 789

115

9

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      10      20      30      40
      *      *      *      *
ATG AAG TAC ATT TTG CTA ATA CTC GCG TGC ATA ATT GCA TGC GTT TAT
TAC TTC ATG TAA AAC GAT TAT GAG CGC ACG TAT TAA CGT ACG CAA ATA
M K Y I L L I L A C I I A C V Y>
a a a a TRANSLATION OF FIPV E1 a a a a >

50      60      70      80      90
*      *      *      *      *
GGT GAA CGC TAC TGT GGC ATG CAA GAC AGT GGC TTG CAG TGT ATT AAT
CCA CTT GCG ATG ACA CGG TAC GTT CTG TCA CCG AAC GTC ACA TAA TTA
G E R Y C A H Q D S G L Q C I ND>
a a a a TRANSLATION OF FIPV E1 a a a a >

100     110     120     130     140
*      *      *      *      *
GGC ACA AAT TCA AGA TGT CAA ACC TGC TTT GAA CGT GGT GAT CTT ATT
CCG TGT TTA AGT TCT ACA GTT TGG ACG AAA CTT GCA CCA CTA GAA TAA
G T N S R C Q T C F E R G D L ID>
a a a a TRANSLATION OF FIPV E1 a a a a >

150     160     170     180     190
*      *      *      *      *
TGG CAT CTT GCT AAC TGG AAC TTC AGC TGG TCT GTA ATA TTG ATT GTT
ACC GTA GAA CGA TTG ACC TTG AAG TCG ACC AGA CAT TAT AAC TAA CAA
W H L A N W N F S W S V I L I V>
a a a a TRANSLATION OF FIPV E1 a a a a >

200     210     220     230     240
*      *      *      *      *
TTT ATA ACA GTG TTA CAA TAT GGC AGA CCA CAA TTT AGC TGG CTC GTT
AAA TAT TGT CAC AAT GTT ATA CCG TCT GGT GTT AAA TCG ACC GAG CAA
F I T V L Q Y G R P Q F S W L V>
a a a a TRANSLATION OF FIPV E1 a a a a >

250     260     270     280
*      *      *      *
TAT GGC ATT AAA ATG CTG ATC ATG TGG CTA TTA TGG CCT ATT GMT CTA
ATA CCG TAA TTT TAC GAC TAG TAC ACC GAT AAT ACC GGA TAA CAA GAT
Y G I K M L I M W L L W P I V LD>
a a a a TRANSLATION OF FIPV E1 a a a a >

290     300     310     320     330
*      *      *      *      *
GCG CTT ACG ATT TTT AAT GCA TAC TCT GAG TAC CAA GTT TOC AGA TAT
CGC GAA TGC TAA AAA TTA CGT ATG AGA CTC ATG GTT CAA AGG TCT ATA
A L T I F N A Y S E Y Q V S R Y>
a a a a TRANSLATION OF FIPV E1 a a a a >

340     350     360     370     380
*      *      *      *      *
GTA ATG TTC GGC TTT AGT GTT GCA GGT GCA GTT GTA ACG TTT GCA CTT
CAT TAC AAG CCG AAA TCA CAA CGT CCA CGT CAA CAT TGC AAA CGT GAA
V M F G F S V A G A V V T F AL>
a a a a TRANSLATION OF FIPV E1 a a a a >

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Figure 1A. Nucleotide and amino acid sequence of FIPV E1 open reading frame.

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390      400      410      420      430
*        *        *        *        *
TGG ATG ATG TAT TTT GTG AGA TCT GTT CAG CTA TAT AGA AGA ACC AAA
AOC TAC TAC ATA AAA CAC TCT AGA CAA GTC GAT ATA TCT TCT TGG TTT
W  M  M  Y  F  V  R  S  V  Q  L  Y  R  R  T  K>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

440      450      460      470      480
*        *        *        *        *
TCA TGG TGG TCT TTT AAT CCT GAG ACT AAT GCA ATT CTT TGT GTT AAT
AGT ACC ACC AGA AAA TTA GGA CTC TGA TTA CGT TAA GAA ACA CAA TTA
S  W  W  S  F  N  P  E  T  N  A  I  L  C  V  ND>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

490      500      510      520
*        *        *        *
GCA TTG GGT AGA AGT TAT GTG CTT CCG TTA GAT GGT ACT CCT ACA GGT
CGT AAC OCA TCT TCA ATA CAC GAA GGG AAT CTA CCA TGA GGA TGT CCA
A  L  G  R  S  Y  V  L  P  L  D  G  T  P  T  G>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

530      540      550      560      570
*        *        *        *        *
GTT ACC CTT ACT CTA CTT TCA GGA AAT CTA TAT GCT GAA GGT TTC AAA
CAA TGG GAA TGA GAT GAA AGT CCT TTA GAT ATA CGA CTT CCA AAG TTT
V  T  L  T  L  L  S  G  N  L  Y  A  E  G  F  K>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

580      590      600      610      620
*        *        *        *        *
ATG GCT GGT GGT TTA ACC ATC GAG CAT TGG OCT AAA TAC GTC ATG ATT
TAC CGA CCA CCA AAT TGG TAG CTC GTA AAC GGA TTT ATG CAG TAC TAA
M  A  G  G  L  T  I  E  H  L  P  K  Y  V  M  ID>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

630      640      650      660      670
*        *        *        *        *
GCT ACA OCT AGT AGA ACC ATC GTT TAT ACA TTA GTT GGA AAA CAA TTA
CGA TGT GGA TCA TCT TGG TAG CAA ATA TGT AAT CAA OCT TTT GTT AAT
A  T  P  S  R  T  I  V  Y  T  L  V  G  K  Q  L>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

680      690      700      710      720
*        *        *        *        *
AAA GCA ACT ACT GOC ACA GGA TGG GCT TAC TAC GTA AAA TCT AAA GCT
TTT CGT TGA TGA CGG TGT CCT ACC CGA ATG ATG CAT TTT AGA TTT CGA
K  A  T  T  A  T  G  W  A  Y  Y  V  K  S  K  A>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

730      740      750      760
*        *        *        *
GGT GAT TAC TCA ACA GAA GCA CGT ACT GAC AAT TTG AGT GAA CAT GAA
CCA CTA ATG AGT TGT CTT CGT GCA TGA CTG TTA AAC TCA CTT GTA CTT
G  D  Y  S  T  E  A  R  T  D  N  L  S  E  H  E>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

770      780
*        *
AAA TTA TTA CAT ATG GTG TAA
TTT AAT AAT GTA TAC CAC APT
K  L  L  H  M  V  ">
TRANSLATION OF FIPV >

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Sequence Range: 1 to 1134

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      10      20      30      40
      *      *      *      *
ATG GCC ACA CAG GGA CAA GGC GTC AAC TGG GGA GAT GAA CCT TCC AAA
TAC CGG TGT GTC OCT GTT GCG CAG TTG ACC OCT CTA CTT GGA AGG TTT
M A T Q G Q R V N W G D E P S K>
a a a a TRANSLATION OF FIPV N a a a a a >

50      60      70      80      90
*      *      *      *      *
AGA CGT GGT CGT TCT AAC TCT CGT GGT CGG AAG AAT AAT GAT ATA CCT
TCT GCA CCA GCA AGA TTG AGA GCA CCA GCC TTC TTA TTA CTA TAT GGA
R R G R S N S R G R K N N D I P>
a a a a TRANSLATION OF FIPV N a a a a a >

100     110     120     130     140
*      *      *      *      *
TTG TCA TTC TAC AAC CCC ATT ACC CTC GAA CAA GGA TCT AAA TTT TGG
AAC AGT AAG ATG TTG GGG TAA TGG GAG CTT GTT OCT AGA TTT AAA ACC
L S F Y N P I T L E Q G S K F W>
a a a a TRANSLATION OF FIPV N a a a a a >

150     160     170     180     190
*      *      *      *      *
AAT TTA TGT CCG AGA GAC CTT GTT CCC AAA GGA ATA GGT AAT AAG GAT
TTA AAT ACA GGC TCT CTG GAA CAA GGG TTT OCT TAT CCA TTA TTC CTA
N L C P R D L V P K G I G N K D>
a a a a TRANSLATION OF FIPV N a a a a a >

200     210     220     230     240
*      *      *      *      *
CAA CAA ATT GGT TAT TGG AAT AGA CAG ATT CGT TAT CGT ATT GTA AAA
GTT GTT TAA CCA ATA ACC TTA TCT GTC TAA GCA ATA GCA TAA CAT TTT
Q Q I G Y W N R Q I R Y R I V K>
a a a a TRANSLATION OF FIPV N a a a a a >

250     260     270     280
*      *      *      *
GGC CAG CGT AAG GAA CTC GCT GAG AGG TGG TTC TTT TAC TTC TTA GGT
COG GTC GCA TTC CTT GAG CGA CTC TCC ACC AAG AAA ATG AAG AAT CCA
G Q R K E L A E R W F F Y F L G>
a a a a TRANSLATION OF FIPV N a a a a a >

290     300     310     320     330
*      *      *      *      *
ACA GGA CCT CAT GCT GAT GCT AAA TTC AAA GAC AAG ATT GAT GGA GTC
TGT OCT GGA GTA CGA CTA CGA TTT AAG TTT CTG TTC TAA CTA OCT CAG
T G P H A D A K F K D K I D G V>
a a a a TRANSLATION OF FIPV N a a a a a >

340     350     360     370     380
*      *      *      *      *
TTC TGG GTT GCA AGG GAT GGT GCC ATG AAC AAG CCC ACA ACG CTT GGC
AAG ACC CAA CGT TCC CTA CCA CGG TAC TTG TTC GGG TGT TGC GAA CCG
F W V A R D G A M N K P T T L G>
a a a a TRANSLATION OF FIPV N a a a a a >

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Figure 1B. Nucleotide and amino acid sequence of FIPV N open reading frame.

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      390      400      410      420      430
      *      *      *      *      *
ACT CGT GGA ACC AAT AAC GAA TOC AAA CCA CTG AGA TTT GAT GGT AAG
TGA GCA CCT TGG TTA TTG CTT AGG TTT GGT GAC TCT AAA CTA CCA TTC

T R G T N N E S K P L R F D G K>
a a a a TRANSLATION OF FIPV N a a a a a >

      440      450      460      470      480
      *      *      *      *      *
ATA CCG CCA CAG TTT CAG CTT GAA GTG AAC CGT TCT AGG AAC AAT TCA
TAT GGC GGT GTC AAA GTC GAA CTT CAC TTG GCA AGA TCC TTG TTA AGT
I P P Q F Q L E V N R S R N N S>
a a a a TRANSLATION OF FIPV N a a a a a >

      490      500      510      520
      *      *      *      *
AGG TCT GGT TCT CAG TCT AGA TCT GTT TCA AGA AAC AGA TCT CAA TCT
TOC AGA CCA AGA GTC AGA TCT AGA CAA AGT TCT TTG TCT AGA GTT AGA
R S G S Q S R S V S R N R S Q S>
a a a a TRANSLATION OF FIPV N a a a a a >

530      540      550      560      570
*      *      *      *      *
AGA GGA AGA CAC CAT TOC AAT AAC CAG AAT AAT AAT GTT GAG GAT ACA
TCT CCT TCT GTG GTA AGG TTA TTG GTC TTA TTA TTA CAA CTC CTA TGT
R G R H H S N N Q N N V E D T>
a a a a TRANSLATION OF FIPV N a a a a a >

580      590      600      610      620
*      *      *      *      *
ATT GTA GGC GTG CTT GAA AAA TTA GGT GTT ACT GAC AAA CAA AGG TCA
TAA CAT CGG CAC GAA CTT TTT AAT CCA CAA TGA CTG TTT GTT TOC AGT
I V A V L E K L G V T D K Q R S>
a a a a TRANSLATION OF FIPV N a a a a a >

630      640      650      660      670
*      *      *      *      *
CGT TCT AAA CCT AGA GAA CGT AGT GAT TOC AAA CCT AGG GAC ACA ACA
GCA AGA TTT GGA TCT CTT GCA TCA CTA AGG TTT GGA TCC CTG TGT TGT
R S K P R E R S D S K P R D T T>
a a a a TRANSLATION OF FIPV N a a a a a >

680      690      700      710      720
*      *      *      *      *
CCT AAG AAT GGC AAC AAA CAC ACC TGG AAG AAA ACT GCA GGC AAG GGA
GGA TTC TTA CGG TTG TTT GTG TGG ACC TTC TTT TGA CGT CCG TTC CCT
P K N A N K H T W K K T A G K G>
a a a a TRANSLATION OF FIPV N a a a a a >

730      740      750      760
*      *      *      *
GAT GTG ACA ACT TTC TAT GGT GCT AGA AGT AGT TCA GCT AAC TTT GGT
CTA CAC TGT TGA AAG ATA CCA CGA TCT TCA TCA AGT CGA TTG AAA CCA
D V T T F Y G A R S S S A N F G>
a a a a TRANSLATION OF FIPV N a a a a a >

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770          780          790          800          810
*           *           *           *           *
GAT AGT GAT CTC GTT GGC AAT GGT AAC GCT GGC AAA TGC TAC CCT CAG
CTA TCA CTA GAG CAA CGG TTA CCA TTG CGA CGG TTT ACG ATG GGA GTC
D S D L V A N G N A A K C Y P Q>
a a a a TRANSLATION OF FIPV N a a a a a >

820          830          840          850          860
*           *           *           *           *
ATA GCT GAA TGT GTT CCA TCA GTG TCT AGC ATA ATC TTT GGC AGT CAA
TAT CGA CTT ACA CAA GGT AGT CAC AGA TCG TAT TAG AAA CCG TCA GTT
I A E C V P S V S S I I F G S Q>
a a a a TRANSLATION OF FIPV N a a a a a >

870          880          890          900          910
*           *           *           *           *
TGG TCT GCT GAA GAA GCT GGT GAT CAA GTG AAA GTC ACG CTC ACT CAC
ACC AGA CGA CTT CTT CGA CCA CTA GTT CAC TTT CAG TGC GAG TGA GTG
W S A E E A G D Q V K V T L T H>
a a a a TRANSLATION OF FIPV N a a a a a >

920          930          940          950          960
*           *           *           *           *
ACC TAC TAC CTG CCA AAG GAT GAT GGC AAA ACT AGT CAA TTC CTA GAA
TGG ATG ATG GAC GGT TTC CTA CTA CGG TTT TGA TCA GTT AAG GAT CTT
T Y Y L P K D D A K T S Q F L E>
a a a a TRANSLATION OF FIPV N a a a a a >

970          980          990          1000
*           *           *           *
CAG ATT GAC GCT TAC AAG CGA CCT TCT GAA GTG GCT AAG GAT CAG AGG
GTC TAA CTG CGA ATG TTC GCT GGA AGA CTT CAC CGA TTC CTA GTC TCC
Q I D A Y K R P S E V A K D Q R>
a a a a TRANSLATION OF FIPV N a a a a a >

1010          1020          1030          1040          1050
*           *           *           *           *
CAA AGA AGA TCC CGT TCT AAG TCT GCT GAT AAG AAG CCT GAG GAG TTG
GTT TCT TCT AGG GCA AGA TTC AGA CGA CTA TTC TTC GGA CTC CTC AAC
Q R R S R S K S A D K K P E E L>
a a a a TRANSLATION OF FIPV N a a a a a >

1060          1070          1080          1090          1100
*           *           *           *           *
TCT GGA ACT CTT GTG GAG GCA TAC ACA GAT GTG TTT GAT GAC ACA CAG
AGA CAT TGA GAA CAC CTC CGT ATG TGT CTA CAC AAA CTA CTG TGT GTC
S V T L V E A Y T D V F D D T Q>
a a a a TRANSLATION OF FIPV N a a a a a >

1110          1120          1130
*           *           *
GTT GAG ATG ATT GAT GAG GTT ACG AAC TAA
CAA CTC TAC TAA CTA CTC CAA TGC TTG ATT
V E M I D E V T N *>
a TRANSLATION OF FIPV N a a >

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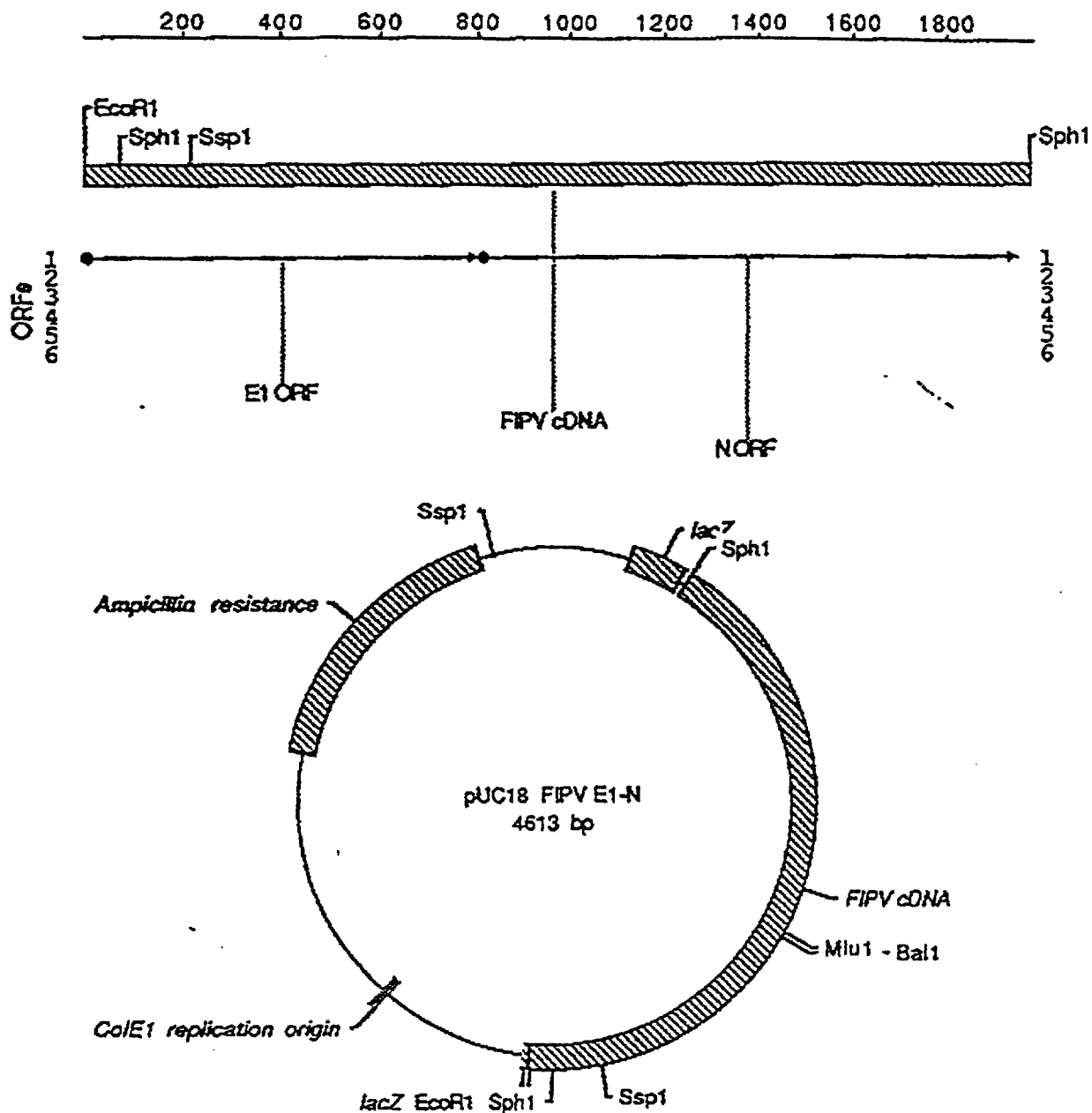


Figure 2. Plasmid pUC18:E1-N and FIPV E1 and N cDNA.

pUC18:E1-N contains a 1.9 kbp EcoRI-SspI fragment containing the FIPV E1 and N genes ligated together.

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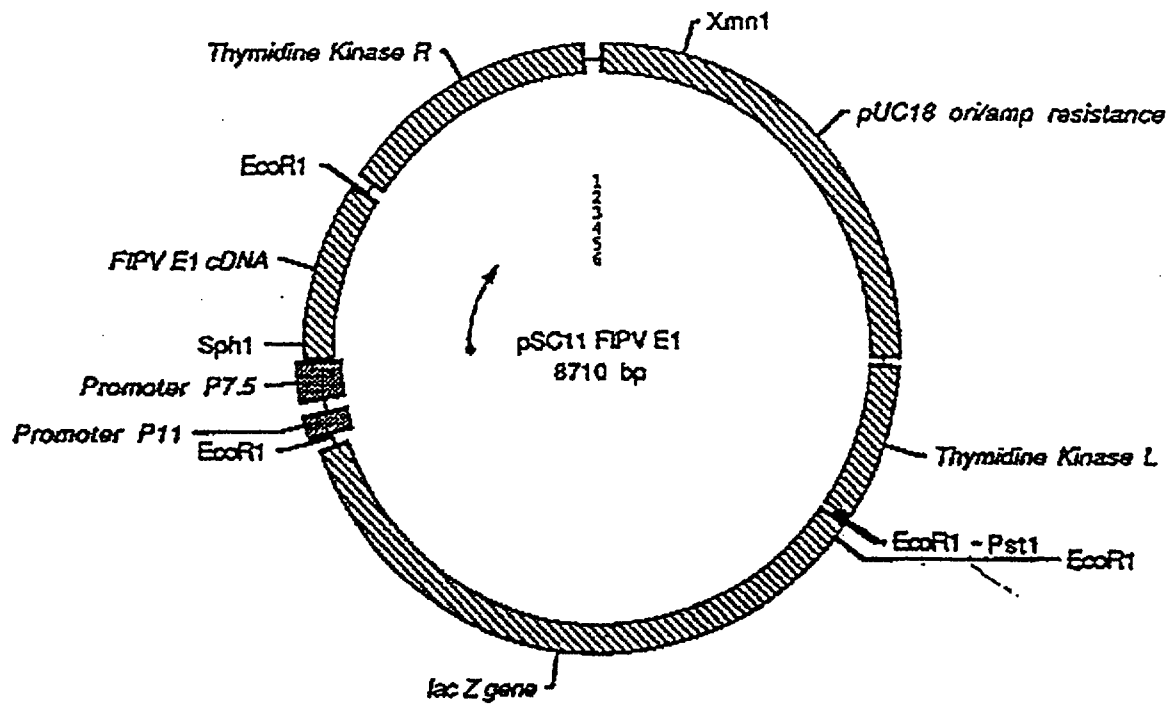


Figure 3B. pSC11 FIPV E1

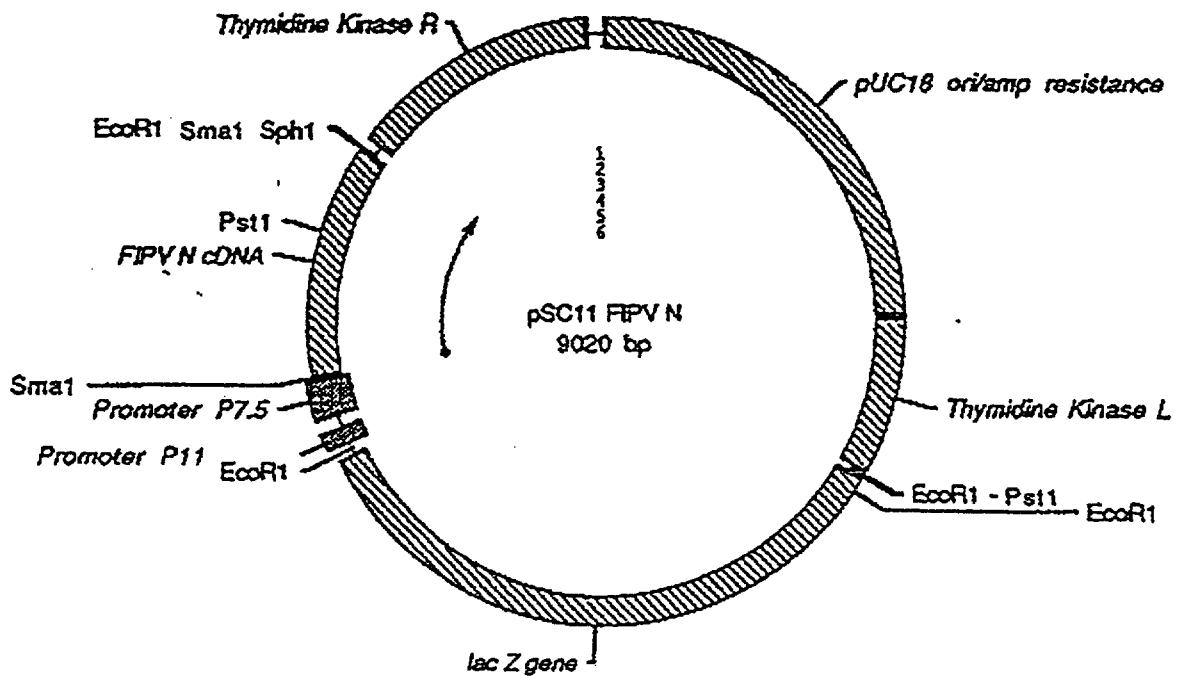


Figure 3C. pSC11 FIPV N

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FEATURES	From	To/Span	Description
DNA	1	2233	pUC18 ori/amp resistance
DNA	7377	8637	Thymidine Kinase R
DNA	6080	3030 (C)	lac Z gene
DNA	2240	3000	Thymidine Kinase L
signal	6250	6130 (C)	Promoter P11
signal	6300	6480	Promoter P7.5
DNA	6493	7329	FIPV E1 cDNA
ORF	6525	7313	1 E1 ORF

1	CGRAAGGGCC	TGCTGATACG	OCTATTTTTA	TAGGTTAATG	TCATGATAAT	AATGGTTTCT
61	TAGACGTCAG	GTGGCACTTT	TGGGGGAAAT	GTGCGGGGAA	COOCTATTTG	TTTATTTTTC
121	TAAATACATT	CAAATATGTA	TOOCTCATG	AGACAATAAC	OCTGATAAAT	GCTTCAATRA
181	TATTGAAAAA	GGAAGAGTAT	GAGTATTCBA	CATTTOOGTG	TGGOCCTTAT	TOOCTTTTTT
241	GCGGCATTTT	GOCCTOCTGT	TTTTGCTCAC	OCAGAAACGC	TGGTGAAAGT	AAABAGTGCT
301	GAAGATCAGT	TGGGTGCAAG	AGTGGGTTAC	ATCGAACTGG	ATCTCAACAG	CGGTAAGTCT
361	CTTGAGAGTT	TTGCGCCCGA	AGAACGTTTT	OCAATGATGA	GCACTTTAA	AGTTCTGCTA
421	TGTGGGCGGG	TATTATCCCG	TATTGACGCC	GGGCAAGAGC	AACTCGGTGG	COGCATACAC
481	TATTCTCAGA	ATGACTTGGT	TGAGTACTCA	CCAGTCACAG	AAAAGCATCT	TACGGTAGGC
541	ATGACAGTAA	GAGAATTATG	CAGTGCTGOC	ATAAOCATGA	GTGATACAC	TGCGGCCAAC
601	TTACTTCTGA	CACGATCGG	AGGACCGAAG	GAGCTAAOCG	CTTTTTTTGCA	CAACATGGGG
661	GATCATGTAA	CTCGCCCTGA	TOGTTGGGAA	COGGAGCTGA	ATGAAGOCAT	ACCAACGAC
721	GAGCGTGACA	CCACGATGCC	TGTAGCAATG	GCAACACOGT	TGCGCAAACT	ATTAACTGGC
781	GAACACTCTA	CTCTAGCTTC	COGGCAACAA	TTAATAGACT	GGATGGAGGC	GGATAAAGTT
841	GCAGGACAC	TTCTGGGCTC	GGOCCTTOCG	GCTGGCTGGT	TTATGTCTGA	TAAATCTGGA
901	GCGGTGTAGC	GTGGGTCTCG	CGGTATCATT	CTAGCACTGG	GGCCAGATGG	TATGCCCTCC
961	CGTATCGTAG	TTATCTACAC	GACGGGGAGT	CAGGCAACTA	TGGATGACCG	AAATAGACAG
1021	ATOGCTGAGA	TAGGTGCTTC	ACTGATTAAAG	CATTGGTAAAC	TGTCAGACCA	AGTTTACTCA
1081	TATATACITT	AGATTGATTT	AAAACITCAT	TTTTAATTTA	AAAGGATCTA	GGTGAAGATC
1141	CTTTTGTATA	ATCTCATGAC	CAAAATOCCT	TAACTGTAGT	TTTCTGTCCA	CTGAGCGTCA
1201	GAOCCCGTAG	AAAAGATCAA	AGGATCTTCT	TGAGATOCCT	TTTTTCTGGG	CGTAACTCTG
1261	TGCTTGCAAA	CAAAAABACC	ACCGCTACCA	GCGGTGGTIT	GTTTGGCCGA	TCAGAGCTA
1321	CCACTCTITT	TTCCGAAGGT	AACTGGCTTC	AGCAGAGCGC	AGATACCAAA	TACTGTCTCT
1381	CTAGTGATAG	CGTAGTTAGG	CCACACTCTC	AGCACTCTCG	TAGCAACGCC	TACATACTCT
1441	GCTCTGCTAA	TCCTGTACCC	AGTGGCTGCT	GCTAGTGGCG	ATAAGTCTGG	TCCTAOCGGG
1501	TTGGAATCAA	GACGATAGTT	ACCGGTTAAG	GCGCAGCGGT	CGGGCTGAC	GCGGGGTTCG
1561	TGCACACAGC	CCAGCTTGGG	GCGAACGAAC	TACACCGAAC	TGAGATAOCT	ACAGCGTGAG
1621	CATTGAGAAA	GCGCAACGCT	TCOCCGAGGG	AGAAAGGCGG	ACAGGTATOC	GGAAGCGGC
1681	AGGGTOGGAA	CAGGAGAGCG	CACGAGGGAG	CTCCAGGGG	GAAACGCGTG	GTATCTTTAT
1741	AGTCTGTGCG	GGTTTCGCGA	OCTCTGACTT	GAGGTGCGAT	TTTTGTGATG	CTGTCAGGG
1801	GGGCGGAGCC	TATGGAAAAA	CGCCAGCAAC	GCGGCTTTT	TACGGTCTCT	GGCTTTTTCG
1861	TGGCTTTTGG	CTCACATGTT	CTTTCTGCG	TTATCCCTCG	ATTCTGTGGA	TACCGTATT
1921	ACCGCTTTTG	AGTGAGCTGA	TACCGCTCGC	CGCAGCGGAA	CGACCGAGCG	CGCGAGTCA
1981	GTGAGCGAGG	AAGCGGAAGA	GCGCCCAATA	CGCAAAACGC	CTCTCCCGCG	GCGTTGGCGG
2041	ATTCAATTAA	GCAGCTGGCA	CGACAGTTT	CCCGACTGGA	AAGCGGGCAG	TGAGCGCAAC
2101	GCAATTAATG	TGAGTGAAGT	CATCTATTAG	GCACCCCGG	CTTACACTT	TATGCTTCOG
2161	GCTCGTATGT	TGTGTGGAAT	TGTGAGCGGA	TAACAATTTC	ACACAGGAAA	CAGCTATGAC
2221	CATGATTACG	CCAGCTTTT	GCGATCAATA	AATGGATCAC	AAOCAGTATC	TCTTAACGAT
2281	GTTCTTTCGA	GATGATGATT	CATTTTTTAA	GTAATTGGCT	AGTCAAGATG	ATGAATCTT
2341	CATTACTTGA	TATATTGCAA	ATCACTCAAT	ATCTAGACTT	TCTGTATTAT	TTATTGATCC
2401	AATCAAAAAA	TAAATTAGAA	GCGTGGGTTC	ATTGTTATGA	ATCTCTTTCA	GAGGAATACA
2461	GACAATTGAC	AAAATTACAA	GACTTTCAAG	ATTTTAAAAA	ACTGTTTAA	AAGGTCCCTA
2521	TTGTTACAGA	TGGAAGGGTC	AAACTTAATA	AAGGATATTT	GTTCGACTTT	GTGATTAGTT
2581	TGATGCGATT	CAAAAAGCAA	TOCTCTCTAG	CTACCAACGC	AATAGATCCT	GTTAGATACA
2641	TAGATCCTCG	TCGCAATATC	GCATTTTCTA	ACGTGATGGA	TATATTAAAG	TCGAATAAAG
2701	TGAACAATAA	TTAATCTTT	ATTGTCATCA	TGAACGGCGG	ACATATTTCAG	TTGATAATCG
2761	GCCCATGTTT	TTCAGGTAAA	AGTACAGAA	TAATTAGACG	AGTTAGACGT	TATCAATAG
2821	CTCAATATAA	ATGCGTGACT	ATAAAATATT	CTAACGATAA	TAGATACGGA	ACGGGACTAT
2881	GGACGCTATG	TAAGAATAAT	TTTGAAGCAT	TGGAAGCAAC	TAAACTATGT	GATCTCTTGG
2941	AATCAATTAC	AGATTCTTCC	GTGATAGGTA	TCGATGAAGG	ACAGTTCTTT	CCAGACTTTG
3001	TTGAAATCCG	AGCTTGGCTG	CAGGTGGGGG	ATCCCCCTTG	CCCGGTTATT	ATTATTTTTG
3061	ACACAGACC	AACTGGTAAT	GATAGCGAAC	GGCGCTCAGC	TGAATTCGCG	CGATACTGAC
3121	GGGCTCCAGG	AGTCGTGCCC	ACCAATCCCC	ATATGGAAAC	CGTCGATATT	CAGCCATGTG

Figure 4A. Nucleotide sequence and location of various genetic elements of pSC11 FIPV E1.

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3181	OCTTCTTCCG	CGTGACGACG	ATGGGATGCG	CTGGTTTCCA	TCAGTTGCTG	TTGACTGTAG
3241	CGGCTGATGT	TGAACGTGAA	GTCGCGCGCG	CAGTGGTGTG	GGCCATAATT	CAATTGCGGC
3301	GTCCCGCAGC	GCAGACCGTT	TTCGCTCGGG	AAGACGTACG	GGGTATACAT	GTCTGACAT
3361	GGCAGATCCG	AGCGGTCAA	ACAGGCGGCA	GTAAGGCGGT	OGGGATAGTT	TTCTTGCGGC
3421	OCTAATCGA	GCCAGTTTAC	COGCTCTGCT	AOCTGCGCCA	GCTGGCAGTT	CAGGOCATC
3481	OGGCGCGGAT	GCGGTGTATC	GCTCGCCACT	TCAACATCAA	CGGTAAATCG	CATTTGACCA
3541	CTACCATCAA	TCCGCTAGGT	TTTCGCGCTG	ATAATTAAGG	TTTTCCCTCG	ATGCTGCCAC
3601	GCGTGACCGG	TGCTAATCAG	CACCGCATCA	GCAAGTGTAT	CTGCGGTGCA	CTGCAACAC
3661	GCTGCTTCGG	CCTGGTAATG	GCGCGCGCGC	TTCAGCGGTT	CGACCCAGGC	GTTAGGGTCA
3721	ATGCGGGTGG	CTTCACTTAC	GCCAATGTGG	TTATCCAGCG	GTGCAAGGGT	GAACTGATCG
3781	CGCAGCGGCG	TCAGCAGTTG	TTTTTTTATG	CCAATCCACA	TCTGTGAAG	AAAGCCTGAC
3841	TGGCGGTAA	ATTGCCBAAG	CTTATTACCC	AGCTCGATGC	AAAAATCCAT	TTCGCTGGTG
3901	GTCCAGATGG	GGATGGCGTG	GGACGCGGCG	GGGAGGTGCA	CAGTGAGGTT	TTCCGCCAGA
3961	CGGCACTGCT	GCCAGCGGCT	GATGTGCCCG	GCCTCTGACC	ATGCGGTGCG	GTTGGTTGCG
4021	ACTACGCGTA	CTGTGAGCCA	GAGTTGCCCG	GCGCTCTCCG	GCTGCGGTAG	TTCAAGCAGT
4081	TCAATCAACT	GTTTAACTTG	TGGAGOGACA	TCCAGAGGCA	CITCAAGCGT	TGCCAGCGGC
4141	TTACCATCCA	GCGCCACCAT	CCAGTGCCAG	AGCTCGTTAT	CGCTATGACG	GAAACAGTAT
4201	TGCGTGGTCA	CITCGATGGT	TTGCCCGGAT	AAAAGGAACT	CGAAAAACTG	CTGCTGGGTT
4261	TTTGCTTCCG	TCAAGCGCTG	ATGCGCGGTG	CGGTGCGCAA	AGACCAAGCC	TTTCAATACG
4321	AACCTGGGAT	CGTTGGGGT	ATCGCCAAA	TCAAGCGCGT	AAGCGGACCA	CGGGTTGGCG
4381	TTTTCATCAT	ATTTAATCAG	CGACTGATCC	ACCCAGTCC	AGACGAGCC	GCCCTGTAAA
4441	CGGCGTACT	GACGAAAGCG	CTGCCAGTAT	TTAGCGAAAC	CGCCAAAGCT	GTTACCATAC
4501	GCGTGGGGCT	ATTGCAAAAG	GATCAGCGGG	CGGCTCTCTC	CAGGTAGCGA	AAGCCATTIT
4561	TTGATGGAAC	ATTGCGGCAC	AGCGGGGAAG	GGCTGGTCTT	CATCCAGCGG	CGCGTACATC
4621	GGGCAATAAA	TATCGGTGGC	CGTGGTGTGG	GCTCGCGCGC	CTTCATACAG	CACCGGGGGG
4681	GAGGATCGA	CAGATTTGAT	CCAGCGATAC	AGCGCGTGGT	GATTAGCGGC	GTGGCGGTAT
4741	TCAATCCCAA	GCGACCGAGT	GATCAGCTCT	GGGTGATTAC	GATCGCGCTG	CACCATTCGC
4801	GTTACGGGTT	CGCTCATCGC	CGGTAGCCAG	CGCGATCAT	CGGTCAAGCG	ATTGATTGGC
4861	ACCATGCGGT	GGGTTTCAAT	ATTGGCTTCA	TCCACCATAT	ACAGGCGGTA	GCGGTGGCAC
4921	AGCGGTACCC	ACAGCGGATG	GTTGCGTAA	TGCGAACAGC	GCAAGCGGTT	AAAGTTGTTC
4981	TGCTTCATCA	CAGGATATC	CTGCAACATC	GTCTGCTCAT	CCATGACCTG	ACCATGCGGA
5041	GGATGATGCT	CGTGACGGTT	AACGCTCTCA	ATCAGCAAGC	GCTTGCGGTT	CAGCAGCAGC
5101	AGACCATTTT	CATCCGCGAC	CTCGCGGAAA	CGGACATCGC	AGGCTTCTGC	TTCAATCAGC
5161	GTGCGGTGG	CGGTGTGCAG	TTCAACACAC	GCAAGATAGA	GATTGCGGAT	TTCGCGGCTC
5221	CACAGTTTCG	GGTTTTCGAC	CTTGAGACGT	AGTGTGACGC	GATGGGCTAT	ACCAACAGCG
5281	TCAATCGATA	TTTACCGGCC	GAAAGGCGCG	GTGCGCGTGG	CGAAGTGGGT	TTCAACCTGC
5341	CATAAGGAAA	CTGTTAOCGG	TAGGTAGTCA	CGCAACTGCG	CGCATATCTG	AACTTCAGCC
5401	TCCAGTACAG	OGGCGCTGAA	ATCATCATTA	AAGCGAGTGG	CAACATGGAA	ATCGCTGATT
5461	TGTGTAGTGG	GTTTATGCAG	CAACGAGACG	TCAAGGAAAA	TGCGGCTCAT	CGGCGACATA
5521	TCTGTATCTT	CCAGATAACT	GCGTCACTC	CAACGACAGC	CCATCAACCG	GAGGCGGTTT
5581	TCTCGGGGCG	GTAATAATGC	GCTCAGGTCA	AATTCAAGCG	GCAAAAGGCT	GTCCTGGCGG
5641	TAAAGGAGCC	AGCGCGCGTT	GCACACAGAG	TGAAAGCGCG	AGTTAAAGCG	ATCAAAAATA
5701	ATTCGCGTCT	GGCTTTCCTG	TAGCCAGCTT	TCAATCAACAT	TAAATGTGAG	CGAGTAACAA
5761	CCGCTCGGAT	TCTCCGTGGG	AACAAAGCGC	GGATTGACCG	TAAATGGGAT	GGTTACGTTG
5821	GTGTAGATGG	GCGCATCGTA	ACCGTGCACT	TGCCAGTTTG	AGGGGACGAC	GACAGTATCG
5881	GCTCAGGAA	GATCGCACTC	CAGCCAGCTT	TCCGGCACCG	CTTCTGGTGC	CGGAAACGAG
5941	GCAAGAGCGC	ATTGCGCATC	CAGGCTGCGC	AAGCTGTGGG	AAGGGCGGTC	GGTGGGGGCC
6001	TCTTCGCTAT	TACGCCAGCT	GGCGAAAGGG	GGATGTGCTG	CAAGGCGATT	AAGTTGGGTA
6061	ACGCCAGGGT	TTTCCAGTGC	ACGACGTTGT	AAAACGACGG	GATCCCTCGA	GGAATTCAAT
6121	TATAGCATAG	AAAAAAACAA	AATGAAATTC	TACTATATTT	TTACATACAT	ATATTCTAAA
6181	TATGAAAGTG	GTGATTGTGA	CTAGCGTAGC	ATCGCTTCTA	GACATATACT	ATATAGTAAT
6241	ACCAATCTCT	AAGACTACGA	AAGTATACCA	ATCTCTTATC	ATGTGGGTAA	TGTTCTCGAT
6301	GTCGATAGC	CATATGCGGG	TAGTTGCGAT	ATACATAAAC	TGATCACTAA	TTCCAAAACC
6361	ACCGGCTTTT	TATAGTAAGT	TTTTCAACCA	TAAATAATTA	ATACATATAT	TAAATTTCTG
6421	TAAAGATAGA	AAATATATTC	TAAATTTATG	CACGGTAAGG	AAGTAGAAGG	ATAGAAACAA
6481	GTGACGGATC	CCAATTGCGG	CATTTTGGGT	TTGAACATAA	CAAAATGAAG	TACATTTTGC
6541	TAAATCTCGC	GTGCATAATT	GCAATGCGTT	ATGGTGAACG	CTACTGTGCC	ATGCAAGACA
6601	GTGGCTTGCA	GTGTATTAA	GGCACAATTC	CAAGATGTCA	AACCTGCTTT	GAAAGTGGTG
6661	ATCTTATTTG	GCATCTTGCT	AAGTGGAACT	TGAGCTGGCT	TGTAATATTTG	ATTGTTTTTA
6721	TAAAGTGTG	ACAATATGGC	AGACCACAAT	TTAGCTGGCT	CGTTTATGGC	ATTAAATATG
6781	TGATCATGTG	GCTATTATGG	CCTATTGTTT	TAGCGCTTAC	GATTTTAAAT	GCACTACTTG
6841	AGTACCAAGT	TTCCAGTAT	GTAATGTTGG	GCCTTAGTGT	TGCAGGTGCA	GTTGTAAAGT
6901	TTGCACCTTG	GATGATGTAT	TTGTGAGAT	CTGTTCAAGT	ATATAGAAGA	ACCAATCAT

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6961 GGTGGTCTTT TAATCCTGAG ACTAATGCAA TTCTTTGTGT TAATGCCATT GGTAGAAATT
7021 ATGTGCTTCC CTTAGATGGT ACTCCTACAG GTGTACCCCT TACTCTACTT TCAGGAAATC
7081 TATATGCTGA AGGTTTCBAA ATGGCTGGTG GTTTAACCAT CGAGCAATTG OCTAABATACG
7141 TCATGATTGC TACACCTAGT AGAACCATCG TTTATACATT AGTTGGABAA CRATTAAHAG
7201 CAACTACTGC CACAGGATGG GCTTACTAOG TAAAAATCTAA AGCTGGTGAT TACTCAACAG
7261 AAGCAOGTAC TGACBATTTG AGTGAACATG AAAAATTATT ACATATGGTG TAACTAAACT
7321 TTCAAATGGG GGAATTCTGT GAGCGTATGG CAAACGAGG AAAAATTAGT TATAGTAGCC
7381 GCACTOGATG GGACATTTCA ACGTAAACCG TTTAATAATA TTTGATCT TATTCCATTA
7441 TCTGAAATGG TGGTAAACT AACTGCTGTG TGTATGAAAT GCCTTAAGGA GGCCTCTTT
7501 TCTAAACGAT TGGGTGAGGA AACCGGATA GRAATATAG GAGGTATGA TATGTATCAA
7561 TCGGTGTGTA GAAAGTGTA CATCGACTCA TAATATTATA TTTTATCT AAAAATCAA
7621 AARTAAACAT TGATTBAATT TTAATATAAT ACTTAAABAT GGATGTTG TGCTTAGATA
7681 AACCGTTTAT GTATTTTGAG GAAATTGATA ATGAGTTAGA TTACGAACCA GRAAGTGCAA
7741 ATGAGGTGCG AAAAAAATG CCGTATCAAG GACAGTTAAA ACTATTTACTA GGAGATTAT
7801 TTTTCTTAG TAAGTTACAG CGACCGGTA TATTAGATGG TGCCACCGTA GTGTATATAG
7861 GATCTGCTCC CGGTACACAT ATACGTTAT TGAAGATCA TTTCTATAT TTAGGATGA
7921 TCATCAAATG GATGCTAATT GACGGGCGC ATCATGATCC TATTTTAAAT GGATTGCGTG
7981 ATGTGACTCT AGTGACTCGG TTOGTTGATG AGSAAATATCT ACGATOCATC AAAAAACAAC
8041 TGCATCCTTC TAAGRTTATT TTAATTTCTG ATGTGAGATC CAAACGAGGA GGAAATGAAC
8101 CTAGTACGGC GGATTTACTA AGTAATTAG CTCTACAAA TGTCAATGAT AGTATTTTAA
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8281 CGTTTTACAA CGTGTGACT GGGAAACOC TGGOGTTACC CAACITATC GOCITGAGC
8341 ACATCCCCCT TTOGOCAGCT GGGGTATAG CGAAGAGGOC CGCAOCGATC GOCITTOCA
8401 ACAGTTGOGC AGOCTGAATG GOGAATGBOG OCTGATGOGG TATTTTCTCT TTACGCATCT
8461 GTGOGGTATT TCACACOGCA TATGGTGAC TCTCAGTACC ATCTGCTCTG ATGOOGCATA
8521 GTTAAGOCAG TACACTOOGC TATOGCTAOG TGACTGGGTC ATGGCTGOGC CCGACACOC
8581 GCAACACOC GCTGACOGC OCTGAOGGC TTGTCTGCTC CCGCATODG CTTACAGACA
8641 AGCTGTGAC GTCTCOGGA GCTGATGTC TCAGAGGTT TCACOGTCAT CAOCGAACOG
8701 CGCGAGGCAG

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FEATURES	From	To/Span	Description
DNA	1	2233	pUC18 ori/amp resistance
DNA	7687	8947	Thymidine Kinase R
DNA	6080	3030 (C)	lac Z gene
DNA	2240	3000	Thymidine Kinase L
signal	6250	6130 (C)	Promoter P11
signal	6300	6480	Promoter P7.5
DNA	6496	7636	FIPV N cDNA
ORF	6496	7629	1 N ORF

1	CGAAAGGGCC	TGCTGATACG	CCTATTTTAA	TAGGTTAATG	TCATGATAAT	AATGGTTTCT
61	TAGACGTCAG	GTGGCACTTT	TGGGGGAAAT	GTGCGCGGAA	CCCCATTTTG	TTTATTTTTC
121	TAAATACATT	CAAAATATGA	TCCGCTCATG	AGACAATAAC	CCTGATAAAT	GCTTCAATAA
181	TATTGAAAAA	GGAAGAGTAT	GAGTATTCAA	CATTTCOCTG	TGGCCCTTAT	TCCCTTTTTT
241	GCGGCATTTT	GCCTTCCTGT	TTTTGCTCAC	CCAGAAACGC	TGGTGAAAGT	AAAAGATGCT
301	GAGATCAGT	TGGGTGCAAG	AGTGGGTTAC	ATCGAAGCTG	ATCTCAACAG	CGGTAGATC
361	CTTGACAGTT	TTCGCCCCGA	AGAAOGTTTT	CCAATGATGA	GCACTTTTAA	AGTTCTGCTA
421	TGTGGCGCGG	TATTATCCCG	TATTGAAGCC	GGGCAAGGAG	AACTCGGTGG	CCGCATACAC
481	TATTCCTCAG	ATGACTTGGT	TGAGTACTCA	CCAGTCACAG	AAAAGCATCT	TACGGATGGC
541	ATGACAGTAA	GAGAATTATG	CAGTCTCGCC	ATACCATGGA	GTGATAACAC	TGCGGCCAAC
601	TTACTCTCTG	CAACGATCGG	AGGACCGRAG	GAGCTBACCG	CTTTTITGCA	CAACATGGGG
661	GATCATGTAA	CTCGCCTTGA	TGTTTGGGAA	CCGGAGCTGA	ATGAAGCCAT	ACCAAACGAC
721	GAGCGTGACA	CCAGCATGOC	TGTAGCAATG	GCAACAACGT	TGCGCAAACT	ATTAAGTGGC
781	GAACTACTTA	CTCTAGCTTC	CCGGCAACAA	TBAATAGACT	GGATGGAGGC	GGATAGAGTT
841	GCAGGACAC	TCTGCGCTTC	GGCCCTTCOG	GCTGGCTGGT	TGATTCCTGA	TAAATCTGGA
901	GCGCGTGGC	GTGGGTCTCG	CGGTATCATT	GCAGCACTGG	GGCCAGATGG	TAGCCCTCC
961	CGTATCGTAG	TTATCTACAC	GACGGGGAGT	CAGGCAACTA	TGGATGAACG	AAATAGACAG
1021	ATCGCTGAGA	TAGGTGCTTC	ACTGATTAG	CATTGGTTAC	TGTCAGACCA	AGTTTACTCA
1081	TATATACCTT	AGATTGATTT	AAAACCTCAT	TTTTAATTTA	AAAGGATCTA	GGTGAAGATC
1141	CTTTTGTATA	ATCTCATGAC	CRAAATCOCT	TAAOGTGAAT	TTTCGTTCCA	CTGAGCGTCA
1201	GACCCCGTAG	AAAAGATCAA	AGGATCTTCT	TGAGATCCTT	TTTTTCGCG	CGTAATCTGC
1261	TGCTTGCAAA	CAAAAABAAOC	ACCGCTTCCA	GCGGTGGTIT	GTTCGCGGA	TCAAGAGCTA
1321	CCACCTCTTT	TTCGGAAGGT	AACTGGCTTC	AGCAGAGCGC	AGTACCAAAA	TACTGTCTCT
1381	CTAGTGTAGC	CGTAGTTAGG	CCACCCTTTC	ARGAATCTCG	TAGCACCOCG	TACATACTTC
1441	GCTCTGTCTA	TCTGTCTAOC	AGTGGCTGCT	GCCAGTGGCG	ATAAGTCTCG	TCTTACCGGG
1501	TTGGACTCAA	GACGATAGTT	ACCGGATAAG	GCGCAGCGGT	CGGGCTGAAC	GGGGGTTTCG
1561	TGCACACAGC	CCAGCTTGGA	GCGAAGGACC	TGCAOOGAAC	TGAGATACTT	ACAGCGTGAG
1621	CATTGAGAAA	GCGCAGCGCT	TCCCGAAGGG	AGAAGGGGGG	ACAGGTATCC	GGTAAGCGGC
1681	AGGGTGGGAA	CAGGAGAGCG	CACGAGGGAG	CTTCCAGGGG	GAAACGCTG	GTATCTTTAT
1741	AGTCTGTGCG	GGTTTGGCCA	CCTCTGACTT	GAGCGTGGAT	TTTTGTGATG	CTCGTCAGGG
1801	GGGCGGAGCC	TATGGAAAAA	CGCCAGCAAC	GCGGCTTTTT	TACGGTTTCT	GGCCTTTTGC
1861	TGGCCTTTTG	CTCACATGTT	CTTTCCTGCG	TTATCCOCTG	ATTCTGTGGA	TAAOCTGATT
1921	ACCGCCTTTG	AGTGAGCTGA	TACCGCTCGC	CGCAGCOGAA	CGACCGAGCG	CAGCGAGTCA
1981	GTGAGCGAGG	AAGCGGAAGA	GCGCCCAATA	CGCAAAOOGC	CTCTCCOOGC	GCGTGTGCOG
2041	ATTCAATTAAT	GCAGCTGGCA	CGACAGGTTT	CCGCACTGGA	AAGCGGGCAG	TGAGCGCAAC
2101	GCAATTAATG	TGAGTTAGCT	CACCTATTAG	GCACCCOAGG	CTTTACACTT	TATGCTTCCG
2161	GCTCGTATGT	TGTGTGGAAT	TGTGAGCGGA	TAAACATTTT	ACACAGGAAA	CAGCTATGAC
2221	CATGATTACG	CCAAGCTTTT	GCGATCAATA	AATGGATCAC	AACCAGTATC	TCTTAAOCTAT
2281	GTTCTTCGCA	GATGATGATT	CATTTTITTA	GTATTTGGCT	AGTCAAGATG	ATGAAATCTT
2341	CATTATCTGA	TATATTGCAA	ATCACTCAAT	ATCTAGACTT	TCGTGTTATTA	TTATGTATCC
2401	AATCAAAAAA	TAAATTAGAA	GCGGTGGGTC	ATTGTTATGA	ATCTCTTTCA	GAGGATACAA
2461	GACAAATGAC	AAAATTCACA	GACTTTCAAG	ATTTTAAAAA	ACTGTTTAAAC	AAGGTCCCTTA
2521	TTGTTACAGA	TGGAAGGGTC	AAACTTAATA	AAGGATATTT	GTTCGACTTT	GTGATTAGTT
2581	TGATGCGATT	CAAAAAAGAA	TCTCTCTAG	CTACCAACGC	AATAGATCCT	GTTAGATACA
2641	TAGATCCTCG	TGCAATATAT	GCAATTTCTA	ACGTGATGGA	TATATTAAAG	TGAAATAAAG
2701	TGAACAATAA	TTAATCTTTT	ATTGTATCTA	TGAACGGCGG	ACATATTCAG	TTGATAATCG
2761	GCCCCATGTT	TTCAGGTAAA	AGTACAGAAT	TAATTAGACG	AGTTAGACGT	TATCAATAG
2821	CTCAATATAA	ATCGGTGACT	ATAAAATATT	CTAACGATAA	TAGATACGGA	ACGGGACTAT
2881	GGACGCATGA	TAAGAATAAT	TTTGAAGCAT	TGGAAGCAAC	TAAACTATGT	GATCTCTTGG
2941	AATCAATTAC	AGATTCTCTC	GATATAGGTA	TOGATGAAGG	ACAGTTCTTT	CCAGACATTG
3001	TGAATTCCG	AGCTTGGCTG	CAGGTGCGGG	ATCCCOCTG	CCCGGTTATT	ATTATTTTTG
3061	ACACCAGACC	AACTGGTAAT	GGTAGCGAAC	GGCGCTCAGC	TGATTTCCGC	CGATACTGAC
3121	GGGCTCCAGG	AGTCGTGCGC	ACCAATCCCC	ATATGGAAC	CGTCGATATT	CAGCCATGTG

Figure 4B. Nucleotide sequence and location of various genetic elements of pSC11 FIPV N.

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3181	CCTTCTTCOG	OGTGCAGCAG	ATGGCGATGG	CTGGTTTCCA	TCAGITGCTG	TTGACTGTAG
3241	CGGCTGATGT	TGAAGTGGAA	GTCGCGOGGC	CACITGGTGTG	GGOCATAATT	CAATTGCGGC
3301	GTCOCGCAGC	GCAGACGGTT	TTGCTCTGGG	AAGACGTACG	GGGTATACAT	GTCGTACAA
3361	GGCAGATCCC	AGCGGTTCARA	ACAGGCGGCA	GTABGGGGGT	CGGGATAGTT	TTCTTGGCGC
3421	CCTAATCCGA	GCCAGTTTAC	COGCTCTGCT	AOCTGOGCCA	GCTGGCAGTT	CAGGCCAATC
3481	CGCGCGGAT	GCGGTGTATC	GCTCGCCACT	TCAACATCAA	CGGTAAATGC	CATTTGAOCC
3541	CTACCATCAA	TCCGGTAGGT	TTTCCGGCTG	ATAAATAAGG	TTTTCCCTCG	ATGCTGCCAC
3601	GCGTGACGGG	TGCTAATCAG	CACCGCATCA	GCAAGTGTAT	CTGOOGTGCA	CTGCAACAAC
3661	GCTGCTTGGG	CCTGGTAAATG	GOCGCGCGGC	TTCCAGCGTT	CGAOCAGGC	GTTAGGGTCA
3721	ATGCGGGTGG	CTTCACTTAC	GOCATGTGCG	TTATCCAGCG	GTGCAOCCGT	GAACGTATCG
3781	OGCAGCGGCG	TCAGCAGTTG	TTTTTTTATCG	CCAATCCACA	TCTGTGAAG	AAAGOCGTAC
3841	TGGCGGTTRA	ATTGCCAAAG	CTTATTACCC	AGCTCGATGC	AAAAATOCAT	TTGCTGGTGT
3901	GTCAGATGCG	GGATGGGGTG	GGACGCGGCG	GGGAGCGTCA	CACITGAGGT	TTCCGGCAGA
3961	CGCCACTGCT	GCCAGGCGCT	GATGTGCCCG	GCTTCTGACC	ATGCGGTGCG	GTTCCGGTTCG
4021	ACTACGGCTA	CTGTAGOGTA	GAGTTGCCCG	GCGCTCTCCG	GCTGCGGTAG	TTGAGGCGAT
4081	TCAATCAACT	GTTTACCTTG	TGGAGGCGCA	TCAGAGGCGA	CTTCAOCCGT	TGCCAGCGGC
4141	TTACCATCCA	GCGCCACCAT	CCAGTGCAGG	AGCTCGTTAT	CGCTATGAG	GAAAGGTAT
4201	TGCTGGTCA	CTTGGATGGT	TTGCCCGGAT	AAACGGAACT	GGAAAGACTG	CTGCTGGTGT
4261	TTTGCCTCCG	TCAGCGCTGG	ATGCGGCGTG	CGGTCCGCAA	AGROCCAGCC	GTTTATACAG
4321	AACTGGCGAT	CGTTGGCGGT	ATCGCCABAA	TCACCGCGGT	AAGCOGACCA	CGGGTGGCG
4381	TTTTCATCAT	ATTTAATCAG	CGACTGATOC	ACCCAGTCCG	AGROGAAAGC	GOCCTGTAAA
4441	CGGGGATACT	GACGAAAGCG	CTGCCAGTAT	TTAGOGAAAC	CGCCAGACT	GTTACCCATC
4501	GCGTGGGGGT	ATTGCGAAAG	GATCGACGGG	CGGCTCTCTC	CAGGTAGCGA	AAGCCATTTT
4561	TTGATGGACC	ATTTGGGCAC	AGCGGGGAG	GGCTGGTCTT	CATCCAGCGG	CGGTATCATC
4621	GGGCAATAAA	TATCGGTGGC	CGTGGTGTGG	GCTCCGCGGC	CTTCAATCTG	CACCGGGGGG
4681	GAAGGATCGA	CAGATTTGAT	CCAGOGATAC	AGOGGTGGT	GATTAGCGCC	GTGGGCTGAT
4741	TCAATCCOCC	GCGACCATAT	GATCACACTC	GGGTGATTTAC	GATCGGCGTG	CAQCATTOGC
4801	GTTACGGGTT	CGCTCATGCG	CGGTAGCCAG	CGGGATCAT	CGGTGAGAG	ATTGATTGGC
4861	ACCATGCGGT	GGGTTTCAAT	ATTGGCTTCA	TCACCCACAT	ACAGGCGGTA	CGGGTCCAC
4921	AGCGTGTAC	ACAGCGGATG	GTTGGGATAA	TGCGAACAGC	GCAAGCGGTT	AAAGTTGTTT
4981	TGCTTCATCA	GCAGGATATC	CTGCACCATC	GCTGCTCAT	CCATGACCTG	ACCATGCAGA
5041	GGATGATGCT	CGTGACGGTT	RACGCTCGA	ATCAGCAAGC	GCTTGGCGTT	CAGCAGCAGC
5101	AGACCATTTT	CAATCCGCAC	CTCGGGGAAA	CCGACATGCG	AGGCTTCGCG	TTCAATCAGC
5161	GTGCGGTGGG	CGGTGTGCAG	TTCAACCAAC	GCAGGTAGA	GATTGGGAT	TTGGGGCTC
5221	CACAGTTTCG	GGTTTTGAC	CTTGAGCGGT	AGTGTGAGCG	GATGGGCATA	ACCAACAGCG
5281	TCATGATAAA	TTTACCGCGC	GAAAGGCGCG	GTGCGGCTGG	CGAOCGTGCT	TTCACTTCGC
5341	CATAAAGAAA	CTGTTACCGG	TAGGTAGTCA	CGCACTGCG	CGCACATCTG	AACTTCAGCC
5401	TCAGTACAG	CGCGGCTGAA	ATCATCTTA	AAGCGAGTGG	CAACATGGAA	ATGCTGTATT
5461	TGTGTATGCG	GTTTATGCG	CAACGAGAGC	TCACGGAAAA	TGCGGCTCAT	CGCCACATA
5521	TOCTGATCTT	CCAGATAACT	GCGTCACTC	CAACGAGCA	CCATCAOCCG	GAGGCGGTTT
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5761	CCCGTGGGAT	TCTCGGTGGG	RACAAACGGC	GGATTGACCG	TAATGGGATA	GGTTACGTTG
5821	GTGTAGATGG	GCGCATGCTA	ACCGTGCATC	TGOCAGTTTG	AGGGGACGAC	GACAGTATCG
5881	GCTCAGGAA	GATCGCACTC	CAGCGAGCTT	TCGGGCAOCC	CTTCTGGTGC	CGGAAACAG
5941	GCAAGCGGCC	ATTGCGCATT	CAGGCTGCGC	AACGTGTTGGG	AAGGGGATC	GGTGGGGGCC
6001	TCTTCGCTAT	TACGCCAGCT	GGCGAAAGGG	GGATGTGCTG	CAAGGOGATT	AAGTTGGGTA
6061	ACGCCAGGGT	TTTCCAGTCA	ACGACGTTGT	AAAACGACGG	GATCCCTCGA	GGAATTCATT
6121	TATAGCATAG	AAAAAAACAA	AATGAAATTC	TACTATATTT	TTACATACAT	ATATTCTAAA
6181	TATGAAAGTG	GATGATGTGA	CTAGCGTAGC	ATCGCTTCTA	GACATATACT	ATATAGTAA
6241	ACCATACTC	AAGACTACGA	AACGTATACA	ATCTCTTATC	ATGTGGGTAA	TGTTCTCGAT
6301	GTCGAATAGC	CATATGCCGG	TAGTTGCGAT	ATACATAAAC	TGATCACTAA	TTCCAAACCC
6361	ACCGGCTTTT	TATAGTAAGT	TTTTCAACCA	TAAATAATAA	ATACATAAAT	TAATTCTCG
6421	TAAAAGTAGA	AAATATATTC	TAATTTATTT	CACGGTAAGG	AAGTAGAATC	ATAAAGAAC
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6601	TACAACCCCA	TTACCCCTGA	ACAAGGATCT	AAATTTTGGA	ATTTATGTCC	GAGAGACCTT
6661	GTTCOCAGAG	GAAATAGGTA	TAAGGATCAA	CAATTTGGTT	ATTGGAATAG	ACAGATTGCT
6721	TATCGTATTG	TAAAGGCCCA	GCGTAAGGAA	CTCGCTGAGA	GGTGGTTCTT	TTACTTCTTA
6781	GGTACAGGAC	CTCATGCTGA	TGCTAAATTC	AAAGACAAGA	TTGATGGAGT	CTTCTGGGTT
6841	GCAAGGGATG	GTGCCATGAA	CAAGCCCAAC	ACGCTTGGCA	CTCGTGGTAC	CAATTAACGAA
6901	TCCAAACCCAC	TGAGATTTGA	TGGTAAGATA	CCGCCACAGT	TTGAGCTTGA	AGTGAACCGT

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7201 ACTGCRGGCA AGGGAGATGT GACAACTTTC TATGGTGCTA GAAGTAGTTC AGCTAACTTT
7261 GGTGATAGTG ATCTCGTTGC CAATGGTAA C GCTGCCAAAT GCTACCOCTA GATAGCTGAA
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7501 AGGCAAAGAA GATCCCGTTC TAAGTCTGCT GATAAGAAGC CTGAGGAGTT GTCTGTAACT
7561 CTTGTGGAGG CATAACAGA TGTGTTTGTG GACACACAGG TTGAGATGAT TGATGAGGTT
7621 ACGAATTAAG OSCATGCCCG GGAATTCCTGT GAGCGTATGG CAAACGAGG AAAAATTAGT
7681 TATAGTAGCC GCACTCGATG GGACATTTCA ACGTAAACCG TTTAATAATA TTTTGAATCT
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7861 TATGTATCAA TCGGTGTGTA GAAAGTGTGA CATCGACTCA TAATATTATA TTTTITATCT
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8461 AGTATTTTAA ACCCGTGGC GTCTAGTCTT AAATGGAGAT GCGCGTTCC AGATCAATGG
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8881 CCGGACACCC GCGAACACCC GCTGACGCGC CCTGBGGGC TTGCTGCTC CCGGCATCCG
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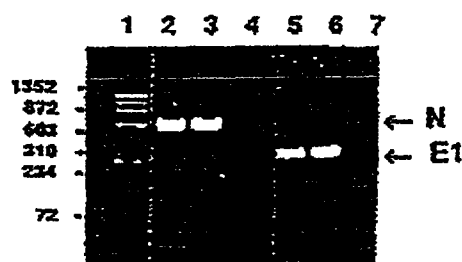


Figure 5. PCR analysis of rRPV-FIPV N and E1 viral-cell lysates.

Lane 1, Base pair DNA markers; Lane 2, pSC11 FIPV N (FIPV N positive control); Lane 3, rRPV-FIPV N; Lane 4, wild-type raccoon pox virus-cell lysates with FIPV N primers (negative control); ; Lane 5, pSC11 FIPV E1 (FIPV E1 positive control); Lane 6, rRPV-FIPV E1; Lane 7, wild-type raccoon pox virus-cell lysates with FIPV E1 primers (negative control).

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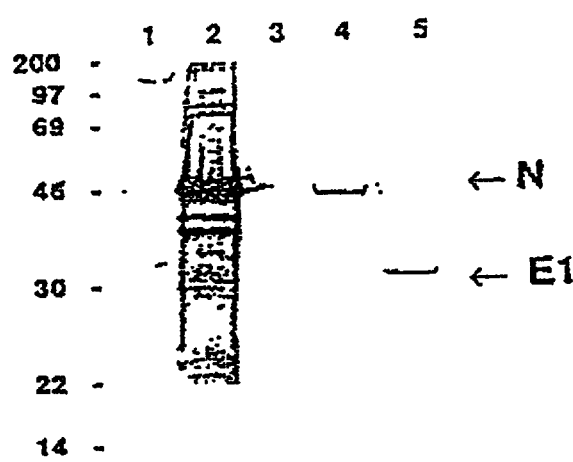


Figure 6. Western blot analysis of rRPV-FIPV N and E1 viral-cell lysates.

Lane 1, molecular weight markers (kDa); Lane 2, purified FIPV antigen (positive control); Lane 3, wild-type raccoon pox virus-cell lysate (negative control); Lane 4, rRPV-FIPV N; Lane 5, rRPV-FIPV E1.

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REPLACEMENT SHEETS		5G
☒	Description	: paged ..1. to paged ..2.
☒	Claim(s)	: paged ..3. to paged ..4.
☒	Drawing(s)	: paged ..5. to paged ..6.
☒	Abstract	: paged ..7. to paged ..8.
DATE OF RECEIPT ..2/12/16.....		

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**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

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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.

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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.)

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.)

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.

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.

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.

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

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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 recombina-
5 torial 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
10 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
15 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
20 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

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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

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

5 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
10 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.

15 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*, *Microsporum canis*, or others. In addition, antigens from the above-cited organisms may be incorporated into combination vaccines. These antigens may be
20 purified from natural sources or from recombinant expression systems, or may comprise individual subunits of the antigen or synthetic peptides derived therefrom.

25 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

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

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(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

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blunt needles attached to a 1 cc syringe, suspended in 0.5 ml of 0.25 µg/ml trypsin, vortexed vigorously, and incubated at 37°C for 15-30 min. The disrupted viral plaques were then inoculated onto 5 x 10⁵ 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 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

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 a monolayer of RAT-2 cells in a 75 cm² flask (approximately 5 x 10⁶ cells) in the presence of 5-bromodeoxyuridine (BrdU) at 30 µg/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 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

The presence of the FIPV N and E1 genes in the RRPVs was confirmed using the polymerase chain reaction (PCR). 90 µl of a virus-cell lysate were incubated with 10 µl 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 µl of this lysate was used in the PCR. PCR was

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performed in 100 μ l of 10 mM Tris-HCL buffer, pH 8.3; 50mM KCl; 200 uM 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:

(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 EI were:

(3) 5'-TATGTAATGTTTCGGCTTTAGTG-3'

(4) 5'-GTGCTTCTGTTGAGTAATCACC-3'

corresponding to nucleotides 334-355 and 721-742 of the FIPV EI 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.

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

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

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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

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(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).

Example 2

PREPARATION OF VACCINE AND TESTING FOR EFFICACY IN CATS

1. Preparation of Master Seeds of RRPV-FIPV N and EI 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

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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

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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:

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SIGN

SCORE

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.

10	Dehydration		1 point/day
	Depression		1 point/day
	Anorexia		1 point/day
	Peritoneal Swelling		1 point/day
	Icterus		1 point/day
	Weight Loss	>20%	1 point per observation
15		>30%	2 points per observation
		>50%	5 points per observation
	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

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.

5

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.

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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	26	77
1272	2	0	0	0	0	0	2
1287	23	0	0	0	34	26	81
1289	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
1306	0	1	0	0	6	0	6
1307	3	1	0	0	4	26	33
1308	2	1	6	0	6	26	40
1311	4	3	0	0	18	26	50
1/10 DOSE SUBCUTANEOUS VACCINATES							
1313	29	5	9	3	36	26	107
1315	17	0	0	0	20	26	62
1317	3	0	3	0	8	26	40
1319	1	0	0	0	6	0	7
CONTROLS							
1321	39	3	18	3	83	26	151
1323	5	0	2	0	14	26	46
1327	0	1	2	0	15	26	43
1329	5	1	6	0	11	26	48
1337	1	0	0	0	0	0	1

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Date: 21 OCT 1996

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

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Date: 21 OCT 1993

(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 ACTGCGTGC ATAATGTCAT GCGTTTATGG TGAACGCTAC	60
TGTGCCATGC AAGACAGTGG CTGCACTGT ATTAATGGCA CAAATTCAAG ATGTCAAACC	120
TGCTTTGAAC GTGGTGATCT TATTGGCAT CTGCTAATC GGAACCTCAG CTGGTCTGTA	180
ATATTGATTG TTTTATAAC AGTGTTACAA TATGCCAGAC CACAAATTAG CTGGCTCGTT	240
TATGGCATTG AATGCTGAT CATGTGGCTA TTATGGCTA TTGTTCTAGC GCTTACGATT	300
TTTAATGTCAT ACTCTGAGTA CCAAGTTTCC AGATATGTAA TGTTOGGCTT TAGTGTTCGA	360
GGTGCACTTG TAACGTTTGC ACTTTGGATG ATGTATTTTG TGAGATCTGT TCAGCTATAT	420
AGAGGAACCA AATCATGGTG GTCITTTAAT CCTGAGACTA ATGCAATTC TGTGTTAAT	480
GCATTGGGTA GAAGTTATGT GCTTCCCTTA GATGGTACTC CTACAGGTGT TACCTTACT	540
CTACTTTCAG GAAATCTATA TGCTGAAGT TTCAAATGG CTGGTGGTTT AACCATOGAG	600
CATTGGCTA AATACGTCAT GATTGCTACA CCTAGTAGAA CCATCGTTTA TACATTAGTT	660
GGAAACAT TAAAGCAAC TACTGCCACA GGATGGGCTT ACTAAGTAA ATCTAAGCT	720
GGTGATTACT CAACAGAGC ACGTACTGAC AATTGAGTG AACATGAAA 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

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Date: 21 OCT 1996

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

ATGGCCACAC AGGGACAAAG CGTCAACTGG GGAGATGAAC CTTCCAAAAG ACGTGGTGGT	60
TCTAACTCTC GTGGTCGGAA GAATAATGAT ATACCTTTGT CATTCTACAA CCCCATTTACC	120
CTCGAACAAAG GATCTAAATT TTGGAATTTA TGTCCGAGAG ACCTTGTTCC CAAAGGAATA	180
GGTAATAAGG ATCAACAAAT TGGTTATTGG AATAGACAGA TTCGTTATCG TATTGTAAAA	240
GGCCAGCGTA AGGAACTCGC TGAGAGGTGG TTCCTTTACT TCCTAGGTAC AGGACCTCAT	300
GCTGATGCTA AATTCAAAGA CAAGATTGAT GGAGTCTTCT GGGTTGCAAG GGATGGTGCC	360
ATGAACAAGC CCACAACGCT TGGCACTCGT GGAACCAATA ACGAATCCAA ACCACTGAGA	420
TTTGATGGTA AGATACCGCC ACAGTTTCAG CTGGAAGTGA ACCGTTCTAG GAACAATTCA	480
AGGTCTGGTT CTCACTCTAG ATCTGTTTCA AGAAACAGAT CTCAATCTAG AGGAAGACAC	540
CATTCCAATA ACCAGAAATA TATGTTGAG GATACAATTG TAGCGTGCT TGAAAATTA	600
GGTGTACAG ACAACAAG GTCACTTCT AAACCTAGAG AACGTAGTA TTCCAACCT	660
AGGGACACAA CACCTAAGAA TGCCAACAA CACACCTGGA AGAAAACGTC AGGCAGGGA	720
GATGTGACAA CTTTCTATGG TGCTAGAAGT AGTTCAGCTA ACTTTGGTGA TAGTGATCTC	780
GTTGCCAATG GTAACGCTGC CAATGCTAC CCTCAGATAG CTGAATGTGT TCCATCAGTG	840
TCTAGCATAA TCCTTGGCAG TCAATGGTCT GCTGAGAAG CTGGTGATCA AGTGAAAGTC	900
ACGCTCACTC ACACCTACTA CCTGCCAAG GATGATGCCA AAACCTAGTCA ATTCTAGAA	960
CAGATTGACG CTTACAAGCG ACCTTCTGAA GTGGCTAAGG ATCAGAGCCA AAGAAGATCC	1020
CGTTCTAAGT CTGCTGATAA GAAGCCTGAG GAGTTGTCTG TAACTCTTGT GGAGGCATAC	1080
ACAGATGTGT TTGATGACAC ACAGGTTGAG ATGATTGATG AGGTTACGAA CTA	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 is the replacement sheet

Date: 21 OCT 1990

(B) CLONE: psc11f1

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

CGAAGGGCC TCGTGATACG CCTATTTTGA TAGGTAAATG TCATGATAAT AATGGTTTCT	60
TAGACGTCCG GTGGCACTTT TGGGGGAAT GTGGCGGGA COCCCTTTTG TTTATTTTTC	120
TAAATACATT CAAATATGTA TCGCTCATG AGACAATAAC CCTGATTAAT GCTTCAATBA	180
TATTGAAAAA GGAAGGTAT GAGTATTCAA CATTTCCGTG TCGCCCTTAT TCCCTTTTTC	240
GCGGCATTTT GCTTCCGTGT TTTTCTCAC CCAGAAAGCC TGGTCAAGT ABAAGATGCT	300
GAGATCAGT TGGGTCCAG AGTGGGTTAC ATCGAAGTGG ATCTCAGCAG CGGTAGATC	360
CTTGAGAGTT TTGCCCCGA AGAAGGTTTT CCAATGATGA GCACTTTTAA AGTCTGTCTA	420
TGTGGGGGGG TATTATCCCG TATTGAGCC GGGCAGAGC AACTCGGTGG CGCATAAC	480
TATCTCAGA ATGACTTGGT TGAATCTCA CCACTCAGC ABAAGCATCT TACGGTGGC	540
ATGACAGTAA GGAATATG CAGTCTGCG ATACCATGA GTGATACAC TCGGCCAAC	600
TTACTCTCA CAACGATGG AGCACGAGG GAGCTACCG CTTTTTGGC CAACATGGG	660
GATCTGTAA CTGCGCTGA TGTGTGGGA CCGGAGCTGA ATGAAGCCAT ACCAAAGAC	720
GAGGTGACA CCGCATGCG TGTAGCAATG GCAACAACT TCGCCAACT ATTAACTGGC	780
GAACTACTTA CTCTAGCTTC CGGCAACAA TTAATAGACT GGATGGAGGC GGTAAAGTT	840
GCAAGACAC TTCTGCGTC GCGCCTCCG GCTGGCTGG TTTTGTCTGA TAAATCTGA	900
CGCGGTGAGC GGGGTCTGG CGGTATCAT GCGGACCTGG GGCCAGATGG TAAGCCCTCC	960
CGTATCGTAG TTATCTACAC GAGGGGGGT CAGGCAACTA TGGATGAACG AATAGACAG	1020
ATGCTGAGA TAGGTGCTTC ACTGATTAG CATTTGTAC TGTGAGACCA AGTTTACTCA	1080
TATBTACTTT AGATTGATTT AAAACTTCAT TTTTATTTA AAGGATCTA GGTGAGATC	1140
CTTTTATATA ATCTCATGAC CAAATCCCT TACGTGAGT TTTGTTCCA CTGAGGCTCA	1200
GACCCGCTAG AAAAGATCAA AGGATCTTCT TCGATCCCT TTTTCTCGG CGTAACTCTG	1260
TGCTTGCAA GAAAAAACC ACGCTACCA GCGGTGCTTT GTTGGCCGA TCAGAGCTA	1320
CCAACTCTTT TTCCGAGGT AACTGGCTTC AGCAGAGGCG AGATACCAA TACTGTCCCT	1380
CTAGTGTAGC CGTAGTGGG CCACCACTTC AAGAACTCTG TAGCAGCGCC TACATACCTC	1440
GCTCTGCTAA TCCGTGACC AGTGGCTGCT GCCAGTGGG ATAAGTCTG TCTTACCGG	1500

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Date: 21 OCT 1985

TTGGACTCAA	GACGATAGTT	ACCGGATAAG	GCTCAGCGGT	CGGGCTGAAC	GGGGGGTTGG	1560
TGCACACAGC	CCAGCTTGGG	GCGACGACC	TACACCGAAC	TGAGATACCT	ACAGCGTGAG	1620
CTTGAGAAA	GCGCCACGCT	TCCCGAAGGG	AGAAAGCGGG	ACAGGTATCC	GGTAAGCGGC	1680
AGGGTCGSA	CAGGAGAGCG	CACGAGGGAG	CTTCCAGGGG	GAAAGCGCTG	GTATCTTTAT	1740
AGTCTGTGG	GGTTTGGCCA	CCCTGACTT	GAGCGTGGAT	TTTTGTGATG	CTGTCAGGG	1800
GGCGGAGCC	TATGGAAAA	CGCGAGCAAC	GCGGCCCTTT	TACGGTTTCT	GGCCTTTTGC	1860
TGGCCTTTTG	CTCACATGTT	CTTCTCTGG	TTATCCCCCG	ATTCTGTGGA	TAACCGTATT	1920
ACCGCCTTTG	AGTGAGCTGA	TACCGCTGCG	CGCAGCGGAA	CGACCGAGCG	CAGCGAGTCA	1980
GTGAGCGAGG	AAGCGGAAGA	GCGGCCAATA	CGCAACCGCG	CTCTCCCCCG	GGCTTGGCGG	2040
ATTCATTAA	GCGCTGGCA	CGCAGGTTT	CCGACTGGA	AAGCGGGCAG	TGAGGSCAAC	2100
GCAATTAAAT	TGAGTTAGCT	CATCTATTAG	GACCCCGAGG	CTTACACTT	TATGCTTCGG	2160
GCTGTATGTT	TGTGTGGAAT	TGTGAGCGGA	TAACCAATTC	ACACAGGAAA	CAGCTATGAC	2220
CTGATTACG	CCAAGCTTTT	GCGTCAATA	AATGGATCAC	AACCAGTATC	TCTTAACGAT	2280
GTCTCTGCA	GATGATGATT	CATTTTTTAA	GTATTGGCT	AGTCAAGATG	ATGAATCTTT	2340
CATTATCTGA	TATATTGCAA	ATCACTCAAT	ATCTAGACTT	TCTGTATATA	TTATTGATCC	2400
AATCAAAAA	TAAATTAGAA	GCGTGGGTC	ATTGTTATGA	ATCTCTTTCA	GAGGAATACA	2460
GACAATTGAC	AAAATTCACA	GACTTTCAG	ATTTAAAAA	ACTGTTTAA	AAGGTCCCTA	2520
TTGTTACAGA	TGGAGGGGTC	AACTTAATA	AAGGATATTT	GTTCGACTTT	GTGATTAGTT	2580
TGATGCGATT	CAAAAAAGAA	TCTCTCTAG	CTACCGCGC	AATAGATCCT	GTAGATACA	2640
TGATCTCTCG	TGCATATATC	GCATTTTCTA	ACGTGATGGA	TATATTAAAG	TGAAATAAG	2700
TGAACAAATA	TTAATTCCTT	ATTGTCTCA	TGAACGGCGG	ACATATTCAG	TGATAATCG	2760
GCCCCATGTT	TTGAGGTAAA	AGTACAGAAT	TAATTAGACG	AGTTAGACCT	TATCAAAATAG	2820
CTCAATATAA	ATGCGTGAAT	ATAAAATATT	CTAACGATAA	TGATACGGA	AAGGGACTAT	2880
GGACGCATGA	TAAAGATAAT	TTTGAAGCAT	TGGAAGCAAC	TAAACTATGT	GATCTCTTGG	2940
AATCAATTAC	AGATTTCTCC	GTGATAGGTA	TGATGAAGG	ACAGTTCTTT	CCAGACATTG	3000
TTGAATTCGG	AGCTTGGCTG	CAGGTGGGGG	ATCCCCCTCG	CCGGTTTATT	ATTATTTTGG	3060
ACCCAGACC	AAGTGGTAAT	GATAGOGAAC	GCGCTCAGC	TGAATTCGCG	CGATCTGAC	3120

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GGGCTCCAGG	AGTGTGCGCC	ACCAATCCCC	ATATGGAAAC	CGTGTATATT	CAGCCATGTG	3180
CCCTCTTCOG	CGTGCAGCAG	ATGGCGATGG	CTGGTTTCCA	TCAGTTGCTG	TTGACTGTAG	3240
CGGCTGATGT	TGAAGTGGAA	GTCGCCGCGC	CACCTGGTGTG	GGCCATAATT	CAATTGCGGC	3300
GTCCCGCAGC	GCAGACCGTT	TTGCTGCGG	AAGACGTACG	GGGTATACAT	GTCTGACAAT	3360
GGCAGATCCC	AGCGGTCAAA	ACAGGCGGCA	GTAAGGCGGT	CGGGATAGTT	TTCTTGCGGC	3420
CCTAATCCGA	GCCAGTTTAC	CGGCTCTGCT	ACCTGCGCCA	GCTGGCAGTT	CAGGCCAATC	3480
CGGCGCGGAT	GCGGTGTATC	GCTGCGCACT	TCAACATCAA	CGGTAATGCG	CATTGACCA	3540
CTACCATCAA	TCCGGTAGGT	TTTCCGGCTG	ATAAATAAGG	TTTTCCCTCG	ATGCTGCCAC	3600
GCGTGACCGG	TGTAATCAG	CACCGCATCA	GCAAGTGTAT	CTGCCGTGCA	CTGCAACRAC	3660
GCTGCCTCGG	CCTGGTAATG	GCCGCGCGCC	TTCCAGCGTT	CGACCCAGGC	GTTAGGGTCA	3720
ATGCGGGTGG	CTTCACTTAC	GCCAATGTGG	TTATCCAGCG	GTGCAAGGGT	GAACTGATCG	3780
CGCAGCGGGG	TCAGCAGTTG	TTTTTTATCG	CCAATCCACA	TCGTGAAAG	AAAGCCTGAC	3840
TGGCGGTTAA	ATTGCCAAGC	CTTATTACCC	AGCTGATGCG	AAAAATCCAT	TTGCTGGTGG	3900
GTCAGATGCG	GGATGGCGTG	GGAGCGCGCG	GGGAGCGTCA	CACCTGAGGT	TTCCGCCAGA	3960
CGCCACTGCT	GCCAGGCGCT	GATGTGCCCG	GCTTCTGACC	ATGCGGTGCG	GTTCGGTTGC	4020
ACTACGCGTA	CTGTGAGCCA	GAGTTGCCCG	GCGCTCTCCG	GCTGCGGTAG	TTGAGGCAGT	4080
TCAATCAACT	GTTTACCTTG	TGGAGCGACA	TCCAGAGGCA	CTTCACCGCT	TGCCAGCGGC	4140
TTACCATCCA	GCGCCACCAT	CCAGTGCGAG	AGCTCGTTAT	CGCTATGACG	GAAACAGGTAT	4200
TGCTGGTCA	CTTCAATGGT	TTGCCCGGAT	AAACGGAACT	GGAAAAACTG	CTGCTGGTGT	4260
TTTGCTTCCG	TCAGCGCTGG	ATGCGGCGTG	CGGTGCGCAA	AGACCAGACC	GTTTATACAG	4320
AACCTGGCGAT	CGTTGCGCGT	ATCGCCAAAA	TCACCGCGGT	AAGCCGACCA	CGGGTTGCCG	4380
TTTTCATCAT	ATTTAATCAG	CGACTGATCC	ACCCAGTCCC	AGACGAGGCC	GCCCTGTAAA	4440
CGGGGATACT	GACGAAACGC	CTGCCAGTAT	TTAGCGAAAC	CGCCAGACT	GTTACCCATC	4500
GCGTGGGCGT	ATTGCAAAAG	GATCAGCGGG	CGGTTCTCTC	CAGGTAGCGA	AAGCCATTTT	4560
TTGATGGACC	ATTTCGGCAC	AGCGGGGAAG	GGCTGGTCTT	CATCCAGCGG	CGGTACATC	4620
GGGCAATAAA	TATCGGTGGC	CGTGGTGTGG	GCTCGCGCGC	CTTCATACTG	CACCGGGCGG	4680
GAAGGATCGA	CAGATTGAT	CCAGCGATAC	AGCGCGTGGT	GATTAGCGCC	GTCGCCGTGAT	4740

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Date: 21 OCT 1995

TCATTCCCCA	GCGACCAGAT	GATCACACTC	GGGTGATTAC	GATCGGCGCG	CACCATTCGC	4800
GTACGGGTT	CGCTCATGCG	CGGTAGCCAG	CGCGGATCAT	CGGTGAGAG	ATTGATTGGC	4860
ACCATGCCGT	GGGTTTCAT	ATTGGCTTCA	TCCACCACAT	ACAGGCCGTA	GCGGTCCAC	4920
AGCGTGTACC	ACAGCGGATG	GTTGGGATAA	TGOGAACAGC	GCACGGGFTT	AAGTTGTTT	4980
TGCTTCATCA	GCAGGATATC	CTGCAOCTC	GTCTGCTCAT	CCATGACCTG	ACCATGCAGA	5040
GGATGATGCT	CGTGACGGTT	AAGGCTCTGA	ATCAGCAACG	GCTTGGCGTT	CAGCAGCAGC	5100
AGACCATTTT	CAATCCGCAC	CTGGCGGAAA	CGGACATCGC	AGGCTTCTGC	TTCATCAGC	5160
GTGCGGTGCG	CGGTGTGCG	TTCACCCACC	GCAOGATAGA	GATTGGGRT	TTOGGGCTTC	5220
CACAGTTTGG	GGTTTTOGAC	CTTGAGAGCT	AGTGTGAGCG	GATCGGCATA	ACCACCAOCC	5280
TCATOGATAA	TTTCACCGCC	GAAAGGCGCG	GTGCGGCTGG	CGACCTGGCT	TTACCCCTGC	5340
CATAAAGAAA	CTGTTACCGG	TAGGTAGTCA	CGCAACTCGC	CGCACATCTG	AACCTTCAGCC	5400
TCCAGTACAG	CGCGGCTGAA	ATCATCTTA	AAGCGAGTGG	CAACATGAAA	ATCGCTGATT	5460
TGTTAGTTCG	GTTTATGCAG	CACGCGAGCG	TCACGGAAAA	TGCGGCTCAT	CGGCCACATA	5520
TCTGTATCTT	CCGATAACT	GCGTCACTC	CAACGCGACA	CCATCACCC	GAGCGCGTTT	5580
TCTCGGCGCG	GTAABAATGC	GCTCGGCTCA	AATTGAGAGC	GCAABOACT	GTCTGGGCGG	5640
TAACCGACCC	AGCGCCCGTT	GCAOCCACGA	TGABAACCGG	AGTTAACGCC	ATCAAAATA	5700
ATTGCGGTCT	GGCCTTCCTG	TAGCGCGCTT	TCATCAACAT	TAAATGTGAG	CGAGTACAA	5760
CCCGTGGGAT	TCTCGGTGGG	AACAAACGGC	GGATTGACCG	TATGGGATA	GTTTACGTTG	5820
GTGTAGATGG	GCGCATCGTA	ACCGTGCATC	TGCCAGTTTG	AGGGGACGAC	GACAGTATCG	5880
GCCTCAGGAA	GATCGCACTC	CAGCAGCTT	TCCGGCACCG	CTTCTGGTGC	CGAAACCG	5940
GCAAGCGGCC	ATTGCGCATT	CAGGCTGCGC	AACGTGTTGG	AAGGCGATC	GGTCCGGGCC	6000
TCTTCGCIAT	TACGCCAGCT	GGCGAABGGG	GGATGTGCTG	CAAGGOSATT	AAGTTEGGTA	6060
ACGCCAGGGT	TTTCCAGTTC	ACGCGTTTGT	AAAACGACGG	GATCCCTCGA	GGAATTCATT	6120
TATAGCATAG	AAAAAACA	AATGAAATTC	TACTATATTT	TTACATACAT	ATATTCTAAA	6180
TATGAAAGTG	GTGATTGTGA	CTAGCGTAGC	ATCGCTTCTA	GACATATACT	ATATAGTAAT	6240
ACCAATACTC	AAGACTACGA	AACGATACA	ATCTCTTATC	ATGTGGGTAA	TGTTCTOGAT	6300
GTCGAATAGC	CATATGCCGG	TAGTTGCGAT	ATACATAAAC	TGATCACTAA	TTCCAAACCC	6360

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Date: 21 OCT 1966

ACCCGCTTTT TATAGTAACT TTTTCACCCA TAAATAATAA ATACATAAAT TAATTTCTCG	6420
TAAAAGTAGA AAATATATTC TAATTTATTC CACGGTAAGG AAGTAGAATC ATAAAGAACA	6480
GTGACGGATC CCAATTCGGG CATTTTGGT TTGAACTAAA CAAATGAAG TACATTTTGC	6540
TAATACTCGC GTGCATAATT GCATGCGTTT ATGGTGAACG CTACTGTGCC ATGCAAGACA	6600
GTGGCTTGCA GTGTATTAAT GGCACAAAT CAGATGTCA AACCTGCTTT GAACGTGGTG	6660
ATCTTATTTG GCATCTGCT AACTGGAAC TCACTGGTC TGTATATTG ATGTTTTAA	6720
TAACAGTGTT ACAAATATGC AGACCACAT TTAGCTGGCT CGTTTATGGC ATTAAATGC	6780
TGATCATGTG GCTATTATCG CCAATTGTT TAGCGCTTAC GATTTTAAAT GCATACTCTG	6840
AGTACCAAGT TTCCAGATAT GTAATGTTG GCTTAGTGT TGCAGGTGCA GTGTAAACGT	6900
TTGCACTTTC GATGATGAT TTTGTGAGAT CTGTTCACT ATATAGAAGA ACCAAATCAT	6960
GGTGGTCTTT TAATCCTGAG ACTAATGCAA TTCTTTGTGT TAATGCATTG GGTAGAAGTT	7020
ATGTGCTTCC CTAGATGTT ACTCCTACAG GTGTACCTT TACTCTACTT TCAGGAATC	7080
TATATGCTGA AGGTTTCAA ATGGCTGGTG GTTAAACCAT CGAGCATTTG CCTAATATCG	7140
TCATGATTGC TACACCTAGT AGAACCATG TTTATACAT MTGTGAAAA CAATTAAAG	7200
CACTACTGTC CACAGGATGG GCTTACTACG TAAATCTAA AGCTGGTGAT TACTCAACAG	7260
AAGCACTTAC TGACAATTTG AGTGAACATG AAAAAATTAT ACATATGGTG TAACATAACT	7320
TTCAAATGGG GGAATTCGT GAGCGTATGG CAAACGAAGG AAAAAATTAGT TATAGTAGCC	7380
GCACTOGATG GGACATTTCA ACGTAAACG TTTAATAATA TTTTGAATCT TATTCATTA	7440
TCTGAAATGG TGGTAAACT AACTGCTGTG TGTATGAAT GCTTAAAGGA GGCTTCTTT	7500
TCTAAACGAT TGGGTGAGGA AACCGAGATA GAAATAATG GAGGTAATGA TATGTATCAA	7560
TGGGTGTGTA GAAAGTGTTA CATCGACTCA TAATATTATA TTTTATCT AAAAACTAA	7620
AAATBAACAT TGATTAAATT TTAATATAAT ACTTAAAAAT GGATGTTGTG TCGTTAGATA	7680
AACCGTTTAT GTATTTTGAG GAAATTGATA ATGAGTTAGA TTACGAACCA GAAAGTGCAA	7740
ATGAGGTGCG AAAAAAACTG CCGTATCAAG GACAGTTAAA ACTATTACTA GGAGAATTAT	7800
TTTTCTTAG TAGTTACAG CGACACGGTA TATTAGATGG TGCCACCGTA GTGTATATAG	7860
GATCTGCTCC CGGTACACAT ATACGTTATT TGAGAGATCA TTTCTATAAT TTAGGAGTGA	7920
TCATCAAATG GATGCTAATT GACGGCCGCC ATCATGATCC TATTTTAAAT GGATTGCGTG	7980

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Date: 21 OCT 1996

ATGTGACTCT AGTGACTGGG TTGTTGATG AGGAATATCT ACGATCCATC AAAAAACAAC	8040
TGCATCCCTC TAAGATTATT TTAATTTCTG ATGTGAGATC CAAACGAGGA GGAAATGAAC	8100
CTAGTACGGC GGATTACTA AGTAATTACG CTCTACAAA TGTCATGATT AGTATTTTAA	8160
ACCCGTTGGC GTCTAGTCTT AAATGGAGAT GCCGTTTCC AGATCAATGG ATCAAGGACT	8220
TTTATATCCC ACACGGTAAT AAAATGTTAC AACCTTTGTC TCCTTCATAT TCAGGGCCGT	8280
CGTTTTACAA CGTCGTGACT GGGAAABCCC TGGCSTTACC CAACTTAATC GCCTTGCAGC	8340
ACATCCCCCT TTGCCCAGCT GGGTAATAG CGAAGAGGCC GGCACOGATC GCCCTTCCCA	8400
ACAGTTGCGC AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCT TTAGCCTCT	8460
GTGCGTATT TCACACOGCA TATGTTGCAC TCTAGTACC ATCTGCTCTG ATGCCGATA	8520
GTTAAGCCAG TACACTCCGC TATOGCTAGG TGACTGGGTC ATGGCTGCGC CCGACACCC	8580
GCCACACCC GCTGAOGGCG CCTGAAGGGC TTGTCTGCTC CCGGCATCG CTTACAGACA	8640
AGCTGTGACC GTCTCGGGA GCTGCATGTG TCAGAGTTT 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

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

GAAAGGGCCT CGTGATAQGC CTATTTTTAT AGGTTAATGT CATGATAATA ATGGTTTCTT	60
AGACGTCAGG TGGCACITTT CGGGGAAATG TGCGGGGAAC CCTATTTTGT TTTATTTTCT	120
AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAT	180
ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT CCCTTTTTGT	240
CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAANGTA AAGATGCTG	300

This is the replacement sheet

Date: 21 OCT 1996

AAGATCAGTT GGGTGCACGA GTGGGTACCA TCGAACTGGA TCTCAACAGC GGTAAGATCC	360
TTGAGAGTTT TCGCCCOGAA GAACGTTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT	420
GTGGCGCGGT ATTATCCOGT ATTGACGCGG GGCAAGAGCA ACTCGGTGCG CGCATACACT	480
ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT ACGGATGGCA	540
TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATACACT GCGGCCAACT	600
TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC TTTTTTGCAC AACATGGGGG	660
ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG	720
AGCGTGACAC CACGATGCCT GTAGCAATGG CAACAACGTT GCGCAACTA TTAACGGCG	780
AACTACTTAC TCTAGCTTCC CGGCAACAT TAAAGACTG GATGGAGGCG GATAAAGTTG	840
CAGGACCACT TCTGCGCTCG GCGCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG	900
CGGTGAGCG TGGGTCTGCG GGTATCATTG CAGCACTGGG GCCAGATGGT AAGCCCTCCC	960
GTATCGTAGT TATCTACAGC ACGGGGAGTC AGGCACTAT GGATGAACGA AATAGACAGA	1020
TGCTGAGAT AGGTGCCTCA CTGATTAGC ATGGCTAAT GTGAGACCA GTTACTCAT	1080
ATATACTTTA GATTGATTTA AAACCTCAT TTTAATTTAA AAGGATCTAG GTGAAGATCC	1140
TTTTGATTA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG	1200
ACCCCTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC GTAATCTGCT	1260
GCTTGCAAC AAAAACAAC CCGCTACCG CGGTGGTTTG TTGCGGGAT CAGAGCTAC	1320
CAACTCTTT TCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAT ACTGTCCTTC	1380
TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAATCTGT AGCAGCGCT ACATACCTCG	1440
CTCTGCTAAT CCTGTACCA GTGGCTGCTG CCAGTGGCGA TAAGTGTGT CTTACCGGT	1500
TGACTCAAG ACGATAGTTA CCGGATAAG CGCAGCGTC GGGCTGAAG GGGGGTTCT	1560
GCACACAGCC CAGCTTGAG CGAACGACCT ACACGAACT GAGATACCTA CAGCGTGAGC	1620
ATTGAGAAAG CGCCACGCTT CCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA	1680
GGTGGGAAC AGGAGAGCGC ACGAGCGAGC TTCCAGGGGG AAACGCTGG TATCTTTATA	1740
GTCTGTGCG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGG	1800
GGCGGAGCCT ATGGA AAAAC GCCAGCAAG CGGCCTTTTT ACGGTTCTTG GCCTTTTGCT	1860
GGCCTTTTGC TCACATGTTT TTTCTGCGT TATCCCTGA TTCTGTGGAT AACCGTATTA	1920

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COGCCCTTGA	GTGAGCTGAT	ACCGCTCGCC	GCAGCCGAAC	GACCGAGCGC	AGOGAGTCRG	1980
TGAGOSAGGA	AGCGGAAGAG	CGCCCAATAC	GCAACCGCC	TCTCCCGCG	CGTTGGCOGA	2040
TTCATTATG	CAGCTGGCAC	GACAGGTTTC	COGACTGGAA	AGCGGGCAGT	GAGCGCAACG	2100
CAATTAATGT	GAGTTAGCTC	ACTCATTAGG	CACCCAGGC	TTCACACTTT	ATGCTTCOGG	2160
CTCGTATGTT	GTGTGGAAAT	GTGAGCGGAT	AACRAATTCA	CACAGGAAAC	AGCTATGACC	2220
ATGATTACGC	CAAGCTTTTG	CGATCAATAA	ATGGATCACA	ACCAGTATCT	CTTAACGATG	2280
TTCCTCGCAG	ATGATGATTC	ATTTTTTAAG	TATTTGGCTA	GTCAAGATGA	TGAAATCTTC	2340
ATTATCTGAT	ATATTGCAAA	TCACTCAATA	TCTAGACTTT	CIGTTATTAT	TATTGATCCA	2400
ATCAAAAAAT	AAATTAGAAG	CGGTGGGTCA	TTGTATGAA	TCTCTTTCAG	AGGAATACAG	2460
ACCAATGACA	AAATTCACAG	ACTTTCAAGA	TTTTAAAAAA	CIGTTTAAAC	AGGTCCCTAT	2520
TGTTACAGAT	GGAGGGGTCA	AACCTAATAA	AGGATATTTC	TTCGACTTTG	TGATTAGTTT	2580
GTTCOGATTC	AAAAAGAAAT	CCTCTCTAGC	TACCACCGCA	ATAGATCCTG	TTAGATACAT	2640
AGATCCTCGT	CGCAATATCG	CATTTTCTAA	CGTGATGGAT	ATATTAAAGT	CGAATAAAGT	2700
GAACAATAAT	TAAATCTTAA	TTGTCAATCAT	GAACGGCGGA	CATATTTCAGT	TGATAATCGG	2760
CCCCATGTTT	TCAAGTAAAA	GTACAGAAAT	AATTAGAAGA	GTTAGACGTT	ATCAAAATGCG	2820
TCAATATATA	TGCGTGACAA	TAAATATTTC	TAAAGTAAAT	AGATACGGAA	CGGGACTATG	2880
GACGCATGAT	AAGAATAAAT	TTGAAGCATT	GGAGGCAACT	AAACTATGTG	ATCTCTTGGA	2940
ATCAATTACA	GATTTCTCCG	TGATAGGTAT	CGATGAAGGA	CAGTTCTTTC	CAGACATTGT	3000
TGAATTCOGA	GCTTGGCTGC	AGGTGGGGGA	TCCCCCCTGC	CCGTTTATTA	TTATTTTTGA	3060
CACCAAGACCA	ACTGGTATTC	GTAGCGAAGC	GCGCTCAGCT	GAATTCGGCC	GATACTGACG	3120
GGCTCCAGGA	GTGTCGCGCA	CCAATCCCCA	TATGGAABACC	GTCGATATTC	AGCCATGTGC	3180
CTTCTTCOGC	GTGCAGCAGA	TGGCGATGGC	TGGTTTCCAT	CAGTTGCTGT	TGACTGTAGC	3240
GGCTGATGTT	GAACTGGAAG	TGCGCGCGCC	ACTGGTGTGG	GCCATAATTC	AATTCOGCGC	3300
TCCCGCAGCG	CAGACCGTTT	TCGCTCGGGA	AGACGTACGG	GGTATACATG	TCTGACAATG	3360
GCAGATCCCA	GCGGTCAAAA	CAGGCGGCAG	TAAGGCGGTC	GGGATAGTTT	TCTTGGCGCC	3420
CTAATCCGAG	CCAGTTTACC	CGCTCTGCTA	CCTGCGCCAG	CTGGCAGTTC	AGGCCAATCC	3480
GCGCCGGATG	CGGTGTATCG	CTCGCCACTT	CAACATCAAC	GGAATCGCC	ATTTGACCAC	3540

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TACCATCAAT	COGGTAGGTT	TTCOGGCTGA	TAAATAAGGT	TTTCCCTGA	TGCTGCCACG	3600
CGTGACCGGT	CGTAATCAGC	ACCGCATCAG	CAAGTGTATC	TGCCGTGCAC	TGCAACAACG	3660
CTGCTTCGGC	CTGGTAATGG	CCCGCCGCTT	TCCAGCGTTC	GACCCAGGCG	TTAGGGTCAA	3720
TGCGGGTCGC	TTCACTTACG	CCAATGTCTG	TATCCAGCGG	TGCACGGGTG	AACTGATCGC	3780
GCAGCGGCGT	CAGCAGTTGT	TTTTTATCGC	CAATCCACAT	CTGTGAAAGA	AAGCCTGACT	3840
GGCGGTTAAA	TTGCCAACGC	TTATTACCCA	GCTCGATGCA	AAAATCCATT	TGCTGGGTGG	3900
TCAGATGCGG	GATGGCGTGG	GACGCGGCGG	GGAGCGTCAC	ACTGAGGTTT	TCGCCAGAC	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	TGCCCCGATA	AACGGAACTG	GAAAACTGC	TGCTGGTGTT	4260
TTGCTTCGGT	CAGCGCTGGA	TGCGGCGTGC	GGTGGGCAA	GACCAAGCCG	TTCTATACAGA	4320
ACTGGCGATC	GTTCGGCGTA	TGCCCCAAAT	CACCGCCGTA	AGCCGACCAC	GGGTTCGGT	4380
TTTCATCAATA	TTTAATCAGC	GACTGATCCA	CCCACTCCCA	GACGAGCCCG	CCCTGTAAAC	4440
GGGGATACTG	ACGAAACGCC	TGCCAGTATT	TAGCGAAACC	GCCAAGACTG	TTACCCATCG	4500
CGTGGGCGTA	TTGCAAAAGG	ATCAGCGGGC	GCGTCTCTCC	AGGTAGCGAA	AGCCATTTTT	4560
TGATGGACCA	TTTGGCACA	GCGGGGAAGG	GCTGGTCTTC	ATCCACGCGC	GCGTACATCG	4620
GGCAAATAAT	ATCGGTGGCC	GTGGTGTGGG	CTCCGCGGCC	TTCTACTGCG	ACCGGGCGGG	4680
AAGGATCGAC	AGATTTGATC	CAGCGATACA	GCGCGTCTGT	ATTAGCGCGG	TGGCCTGATT	4740
CATTCCCCAG	CGACCAAGATG	ATCACACTCG	GGTGATTACG	ATCGCGCTGC	ACCATTCGCG	4800
TTACGCGTTC	GCTCATCGCC	GGTAGCCAGC	GCGGATCATC	GGTCAGACGA	TTGATTGGCA	4860
CCATGCGGTG	GGTTTCAATA	TTGGCTTCAT	CCACCACATA	CAGGCGGTAG	CGGTGCGACA	4920
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GCTTCATCAG	CAGGATATCC	TGCACCATCG	TCTGCTCATC	CATGACCTGA	CCATGCAGAG	5040
GATGATGCTC	GTGACGGTTA	ACGCCTCGAA	TCAGCAACGG	CTTGCCGTTT	AGCAGCAGCA	5100
GACCATTTTT	AATCCGCACC	TGCGGGAAC	CGACATCGCA	GGCTTCTGCT	TCAATCAGCG	5160

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TGCCGTCCGC	GGTGTGCACT	TCAACCACCG	CACGATAGAG	ATTCCGGGATT	TCCGGCGCTCC	5220
ACAGTTTCGG	GTTFTCGACC	TGAGACGTA	GTGTGACGG	ATCCGGCATAA	CCACCACGCT	5280
CATCGATAAT	TTACCGCCG	AAAGGCGCG	TGCCGCTGGC	GACCTGCGTT	TCACCCCTGCC	5340
ATAAAGAAAC	TGTTACCCGT	AGGTAGTCAC	GCAACTCGCC	GCACATCTGA	ACTTCAGCCT	5400
CCAGTACAGC	GCGGCTGAAA	TCATCATTAA	AGCGAGTGGC	AACATGGAAA	TGCTGATTT	5460
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CCTGATCTTC	CAGATAACTG	CCGTCACTCC	AACGACGAC	CATCACCGCG	AGGCGGTTTT	5580
CTCCGGCGCG	TAAAAATGCG	CTCAGGTCAA	ATTACAGCGG	CAACGACTG	TCCTGGCGGT	5640
AACCGACCCA	GCGCCCGTTG	CACCAAGAT	GAAACGCGA	GTTAAGCCA	TCAAAAATAA	5700
TTCCGCTCTG	GCCCTCCTGT	AGCCAGCTTT	CATCAACATT	AAATGTGAGC	GAGTAACAAC	5760
CCGTCCGATT	CTCCGTGGGA	ACAAACGGCG	GATTGACCGT	AATGGGATAG	GTTACGTTGG	5820
TGTAGATGGG	CGCATCGTAA	CCGTGCACT	GCCAGTTTGA	GGGGACGACG	ACAGTATCGG	5880
CCTCAGGAAG	ATCGCACTCC	AGCCAGCTTT	CCGGCACCGC	TTCTGGTGCC	GGAAACCGG	5940
CAAAGCGCCA	TTCCGCTTC	AGGCTGCGCA	ACTGTTGGGA	AGGGCGATCG	GTGCGGCGCT	6000
CTTCGCTATT	ACGCCAGCTG	GCGAAAGGGG	GATGTGCTGC	AAGGCGATTA	AGTTGGGTAA	6060
CGCCAGGGTT	TTCCCACTCA	CGAGTTGTA	AAACGACGGG	ATCCCTCGAG	GAATTCAATT	6120
ATAGCATAGA	AAAAAACAAA	ATGAAATCT	ACTATATTTT	TACATACATA	TATTCTAANT	6180
ATGAAAGTGG	TGATTGTGAC	TAGCGTAGCA	TGCTTCTAG	ACATATACTA	TATAGTATA	6240
CCATACTCA	AGACTACGAA	ACTGATACAA	TCTCTTATCA	TGTGGGTAAAT	GTTCTCGATG	6300
TCGAATAGCC	ATATGCCGGT	AGTTGCGATA	TACATAAAT	GATCACTAAT	TCCAAACCCA	6360
CCCGCTTTTT	ATAGTAAGTT	TTTCACCCAT	AAATAATAAA	TACAATAATT	AATTTCTCGT	6420
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TGACGGATCC	CGGGATGGCC	ACACAGGAC	AAACGCTCAA	CTGGGGAGAT	GAACCTTCCA	6540
AAAGACGTGG	TCGTTCTAAC	TCTCGTGGTC	GGAAGAATAA	TGATATACTT	TTGTCACTCT	6600
ACAACCCCAT	TACCCCTCGAA	CAAGGATCTA	AATTTTGGAA	TTTATGTCCG	AGAGACCTTG	6660
TTCCCAAAGG	AATAGGTAAT	AAGGATCAAC	AAATTGGTTA	TTGGAATAGA	CAGATTGCTT	6720
ATCGTATTGT	AAAAGGCCAG	CGTAAGGAAC	TGCTGAGAG	GTGGTTCTTT	TACTTCTTAG	6780

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GTACAGGACC	TCATGCTGAT	GCTAATTTCA	AAGACAAGAT	TGATGGAGTC	TTCTGGGTTG	6840
CAAGGGATGG	TGCCATGAAC	AAGCCCACAA	CGCTTGGCAC	TCGTGGAACC	AATAACGAAT	6900
CCAAACCACT	GAGATTTGAT	GGTAAGATAC	CGCCACAGTT	TCAGCTTGAA	GTGAACCGTT	6960
CTAGGAACAA	TTCAAGGTCT	GGTTCTCAGT	CTAGATCTGT	TTCAAGAAAC	AGATCTCAAT	7020
CTAGAGGAAG	ACACCATTC	AATAACCAGA	ATAATAATGT	TGAGGATACA	ATTGTAGCCG	7080
TGCTTGAAAA	ATTAGGTGTT	ACTGACAAAC	AAAGGTCACG	TTCTAAACCT	AGAGAACGTA	7140
GTGATTCCAA	ACCTAGGGAC	ACAACACCTA	AGAATGCCAA	CAAACACACC	TGGAAGAAAA	7200
CTGCAGGCAA	GGGAGATGTG	ACAACTTTCT	ATGGTGCTAG	AAGTAGTTCA	GCTAACTTTG	7260
GTGATAGTGA	TCTCGTTGCC	AATGGTAAAG	CTGCCAATG	CTACCOCTCAG	ATAGCTGAAT	7320
GTGTTCCATC	AGTGTCTAGC	ATAATCTTTG	GCAGTCAATG	GTCTGCTGAA	GAAGCTGGTG	7380
ATCAAGTGAA	AGTCACGCTC	ACTCACACCT	ACTACCTGCC	AAAGGATGAT	GCCAAAACCTA	7440
GTCAATTCCT	AGAACAGATT	GACGCTTACA	AGCGACCTTC	TGAAGTGGCT	AAGGATCAGA	7500
GGCAAGGAAG	ATCCCGTTCT	AAGTCTGCTG	ATAAGAAGCC	TGAGGAGTTG	TCTGTAACCTC	7560
TTGTGGAGGC	ATACACAGAT	GTGTTTGTATG	ACACACAGGT	TGAGATGATT	GATGAGGTTA	7620
CGAACTAAAC	GCAATGCCCG	GAATTCGTGTG	AGCGTATGGC	AAACGAAGGA	AAAATTAGTT	7680
ATAGTAGCCG	CACCTGATGG	GACATTTCAA	CGTAAACCGT	TATATAATAT	TTTGAATCTT	7740
ATTCCATTAT	CTGAATGGT	GGTAAACTA	ACTGCTGTGT	GTATGAATG	CTTTAAGGAG	7800
GCTTCCTTTT	CTAAACGATT	GGGTGAGGAA	ACCGAGATAG	AAATAATAGG	AGGTAAATGAT	7860
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AAAAACTAAA	AATAAACATT	GATTAAATTT	TATATATATA	CTTAAAAATG	GATGTTGTGT	7980
CGTTAGATAA	ACCGTTTATG	TATTTTGAGG	AAATTGATAA	TGAGTTAGAT	TACGAACCAG	8040
AAAGTGCAAA	TGAGGTGCGA	AAAAAACTGC	CGTATCAAGG	ACAGTTAAAA	CTATTACTAG	8100
GAGAAATTAT	TTTTCTTAGT	AAGTTACAGC	GACACGGTAT	ATTAGATGGT	GCCACCGTAG	8160
TGTATATAGG	ATCTGCTCCC	GGTACACATA	TACGTTATTT	GAGAGATCAT	TTCTATAATT	8220
TAGGAGTGAT	CATCAATGG	ATGCTAATTG	AAGGCCGCCA	TCATGATCCT	ATTTTAAATG	8280
GATTGCGTGA	TGTGACTCTA	GTGACTGGT	TCGTTGATGA	GGAATATCTA	CGATCCATCA	8340
AAAAACAACCT	GCATCCTTCT	AAGATTATTT	TAATTTCTGA	TGTGAGATCC	AAACGAGGAG	8400

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GAAATGAACC TAGTACGGCG GATTIACIAA GTAATTACGC TCTACAAAAT GTCATGATTA	8460
GTATTTTAAA CCCCGTGGCG TCTAGTCTTA AATGGAGATG CCGGTTTCCA GATCAATGGA	8520
TCAAGGACTT TTATATCCCA CACGGTAATA AAATGTTACA ACCTTTGTCT CCTTCATATT	8580
CAGGGCCGTC GTTTTACAAC GTGCTGACTG GGAAAACCCCT GGCGTTACCC AACTTAATCG	8640
CCTTGCAACA CATCCCCCTT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG	8700
CCCTTCCCAA CAGTTGCGCA GCGTGAATGG CGAATGGCGC CTGATGCGGT ATTTTCTCTT	8760
TACGATCTG TCGGGTATTT CACACCGCAT ATGGTGCACT CTCAGTACCA TCTGCTCTGA	8820
TGCOSCATAG TTAAGCCAGT ACACTCOGCT ATGCTACGT GACTGGGTCA TGGCTGCGCC	8880
CCGACACCCG CCAACACCCG CTGACGCGCC CTGACGGGCT TGTCTGCTCC CGGCATCCGC	8940
TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCTGTGT CAGAGGTTTT CACCGTCATC	9000
ACCGAAACGC GCGAGGCAG	9019

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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

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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

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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.

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ABSTRACT

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

The invention that relates to recombinant raccoon poxvirus useful as a Vaccine Against Feline Infectious Peritonitis Virus Disease. The recombinant raccoon poxvirus has 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).

NO FIGURE

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Date: 23 OCT 1986

sequence range:
1 to 789

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      10          20          30          40
      *          *          *          *
ATG AAG TAC ATT TTG CTA ATA CTC GCG TGC ATA ATT GCA TGC GTT TAT
TAC TTC ATG TAA AAC GAT TAT GAG CGC ACG TAT TAA CGT ACG CAA ATA
M  K  Y  I  L  L  I  L  A  C  I  I  A  C  V  Y>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

50          60          70          80          90
*          *          *          *          *
GGT GAA CGC TAC TGT GCC ATG CAA GAC AGT GGC TTG CAG TGT ATT AAT
CCA CTT GCG ATG ACA CGG TAC GTT CTG TCA CCG AAC GTC ACA TAA TTA
G  E  R  Y  C  A  M  Q  D  S  G  L  Q  C  I  N>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

100         110         120         130         140
*          *          *          *          *
GGC ACA AAT TCA AGA TGT CAA ACC TGC TTT GAA CGT GGT GAT CTT ATT
CCG TGT TTA AGT TCT ACA GTT TGG ACG AAA CTT GCA CCA CTA GAA TAA
G  T  N  S  R  C  Q  T  C  F  E  R  G  D  L  I>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

150         160         170         180         190
*          *          *          *          *
TGG CAT CTT GCT AAC TGG AAC TTC AGC TGG TCT GTA ATA TTG ATT GTT
ACC GTA GAA CGA TTG ACC TTG AAG TGG ACC AGA CAT TAT AAC TAA CAA
W  H  L  A  N  W  N  F  S  W  S  V  I  L  I  V>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

200         210         220         230         240
*          *          *          *          *
TTT ATA ACA GTG TTA CAA TAT GGC AGA CCA CAA TTT AGC TGG CTC GTT
AAA TAT TGT CAC AAT GTT ATA CCG TCT GGT GTT AAA TGG ACC GAG CAA
F  I  T  V  L  Q  Y  G  R  P  Q  F  S  W  L  V>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

250         260         270         280
*          *          *          *
TAT GGC ATT AAA ATG CTG ATC ATG TGG CTA TTA TGG CCT ATT GTT CTA
ATA CCG TTA TTT TAC GAC TAG TAC ACC GAT AAT ACC GGA TAA CAA GAT
Y  G  I  K  M  L  I  M  W  L  L  W  P  I  V  L>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

290         300         310         320         330
*          *          *          *          *
GCG CTT ACG ATT TTT AAT GCA TAC TCT GAG TAC CAA GTT TCC AGA TAT
CGC GAA TGC TAA AAA TTA CGT ATG AGA CTC ATG GTT CAA AGG TCT ATA
A  L  T  I  F  N  A  Y  S  E  Y  Q  V  S  R  Y>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

340         350         360         370         380
*          *          *          *          *
GTA ATG TTC GGC TTT AGT GTT GCA GGT GCA GTT GTA ACG TTT GCA CTT
CAT TAC AAG CCG AAA TCA CAA CGT CCA CGT CAA CAT TGC AAA CGT GAA
V  M  F  G  F  S  V  A  G  A  V  V  T  F  A  L>
a  a  a  a  TRANSLATION OF FIPV E1 a  a  a  a  a  >

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Figure 1A

Nucleotide and amino acid sequence of FIPV E1 open reading frame.

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Date:

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390          400          410          420          430
*           *           *           *           *
TGG ATG ATG TAT TTT GTG AGA TCT GTT CAG CTA TAT AGA AGA ACC AAA
BOC TAC TAC ATA AAA CAC TCT AGA CAA GTC GAT ATA TCT TCT TGG TTT
W  M  M  Y  F  V  R  S  V  Q  L  Y  R  R  T  K>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

440          450          460          470          480
*           *           *           *           *
TCA TGG TGG TCT TTT AAT CCT GAG ACT AAT GCA ATT CTT TGT GTT AAT
AGT ACC ACC AGA AAA TTA GGA CTC TGA TTA CGT TAA GAA ACA CAA TTA
S  W  W  S  F  N  P  E  T  N  A  I  L  C  V  N>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

490          500          510          520
*           *           *           *
GCA TTG GGT AGA AGT TAT GTG CTT CCC TTA GAT GGT ACT OCT ACA GGT
CGT AAC CCA TCT TCA ATA CAC GAA GGG AAT CTA CCA TGA GGA TGT CCA
A  L  G  R  S  Y  V  L  P  L  D  G  T  P  T  G>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

530          540          550          560          570
*           *           *           *           *
GIT AOC CTT ACT CTA CTT TCA GGA AAT CTA TAT GCT GAA GGT TTC AAA
CAA TGG GAA TGA GAT GAA AGT OCT TTA GAT ATA CGA CTT CCA AAG TTT
V  T  L  T  L  L  S  G  N  L  Y  A  E  G  F  K>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

580          590          600          610          620
*           *           *           *           *
ATG GCT GGT GGT TTA ACC ATC GAG CAT TTG OCT AAA TAC GTC ATG ATT
TAC CGA CCA CCA AAT TGG TAG CTC GTA AAC GGA TTT ATG CAG TAC TAA
M  A  G  G  L  T  I  E  E  L  P  K  Y  V  M  D>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

630          640          650          660          670
*           *           *           *           *
GCT ACA OCT AGT AGA ACC ATC GTT TAT ACA TTA GTT GGA AAA CAA TTA
CGA TGT GGA TCA TCT TGG TAG CAA ATA TGT AAT CAA OCT TTT GTT AAT
A  T  P  S  R  T  I  V  Y  T  L  V  G  K  Q  L>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

680          690          700          710          720
*           *           *           *           *
AAA GCA ACT ACT GGC ACA GGA TGG GCT TAC TAC GTA AAA TCT AAA GCT
TTT CGT TGA TGA CGG TGT OCT ACC CGA ATG ATG CAT TTT AGA TTT CGA
K  A  T  T  A  T  G  W  A  Y  Y  V  K  S  K  B>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

730          740          750          760
*           *           *           *
GGT GAT TAC TCA ACA GAA GCA CGT ACT GAC AAT TTG AGT GAA CAT GAA
CCA CTA ATG AGT TGT CTT CGT GCA TGA CTG TTA AAC TCA CTT GTA CTT
G  D  Y  S  T  E  A  R  T  D  N  L  S  E  E  E>
a  a  a  a  TRANSLATION OF FIPV El a  a  a  a  a  >

770          780
*           *
AAA TTA TTA CAT ATG GTG TAA
TTT AAT AAT GTA TAC CAC ATT
K  L  L  H  M  V  *>
TRANSLATION OF FIPV >

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sequence range:
1 to 1134

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      10      20      30      40
      *      *      *      *
ATG GGC ACA CAG GGA CAA GGC GTC AAC TGG GGA GAT GAA CCT TCC AAA
TAC CGG TGT GTC CCG GTT GCG CAG TTG ACC CCT CTA CTT GGA AGG TTT
M  A  T  Q  G  Q  R  V  N  W  G  D  E  P  S  K>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

50      60      70      80      90
      *      *      *      *
AGA CGT GGT CGT TCT AAC TCT CGT GGT CGG AAG AAT AAT GAT ATA CCT
TCT GCA CCA GCA AGA TTG AGA GCA CCA GCC TTC TTA TTA CTA TAT GGA
R  R  G  R  S  N  S  R  G  R  K  N  N  D  I  P>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

100     110     120     130     140
      *      *      *      *
TTG TCA TTC TAC AAC CCG ATT ACC CTC GAA CAA GGA TCT AAA TTT TGG
AAC AGT AAG ATG TTG GGG TAA TGG GAG CTT GTT CCG AGA TTT AAA ACC
L  S  F  Y  N  P  I  T  L  E  Q  G  S  K  F  W>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

150     160     170     180     190
      *      *      *      *
AAT TTA TGT CCG AGA GAC CTT GTT CCG AAA GGA ATA GGT AAT AAG GAT
TTA AAT ACA GGC TCT CTG GAA CAA GGG TTT CCG TAT CCA TTA TTC CTA
N  L  C  P  R  D  L  V  P  K  G  I  G  N  K  D>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

200     210     220     230     240
      *      *      *      *
CAA CAA ATT GGT TAT TGG AAT AGA CAG ATT CGT TAT CGT ATT GTA AAA
GTT GTT TAA CCA ATA ACC TTA TCT GTC TAA GCA ATA GCA TAA CAT TTT
Q  Q  I  G  Y  W  N  R  Q  I  R  Y  R  I  V  K>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

250     260     270     280
      *      *      *      *
GGC CAG CGT AAG GAA CTC GCT GAG AGG TGG TTC TTT TAC TTC TTA GGT
CCG GTC GCA TTC CTT GAG CGA CTC TOC ACC AAG AAA ATG AAG AAT CCA
G  Q  R  K  E  L  A  E  R  W  F  F  Y  F  L  G>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

290     300     310     320     330
      *      *      *      *
ACA GGA CCT CAT GCT GAT GCT AAA TTC AAA GAC AAG ATT GAT GGA GTC
TGT CCG GGA GTA CGA CTA CGA TTT AAG TTT CTG TTC TAA CTA CCG CAG
T  G  P  H  A  D  A  K  F  K  D  K  I  D  G  V>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

340     350     360     370     380
      *      *      *      *
TTC TGG GTT GCA AGG GAT GGT GCC ATG AAC AAG CCC ACA ACG CTT GGC
AAG ACC CAA CGT TOC CTA CCA CCG TAC TTG TTC GGG TGT TGC GAA CCG
F  W  V  A  R  D  G  A  M  N  K  P  T  T  L  G>
a  a  a  a  TRANSLATION OF FIPV N a a a a a >

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Figure 1B

Nucleotide and amino acid sequence of FIPV N open reading frame.

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390      400      410      420      430
*      *      *      *      *
ACT CGT GGA ACC AAT AAC GAA TCC AAA CCA CTG AGA TTT GAT GGT AAG
TGA GCA OCT TGG TTA TTG CTT AGG TTT GGT GAC TCT AAA CTA CCA TTC

T R G T N N E S K P L R F D G K>
a a a a TRANSLATION OF FIPV N a a a a a >

440      450      460      470      480
*      *      *      *      *
ATA CCG CCA CAG TTT CAG CTT GAA GTG AAC CGT TCT AGG AAC AAT TCA
TAT GGC GGT GTC AAA GTC GAA CTT CAC TTG GCA AGA TCC TTG TTA AGT
I P P Q F Q L E V N R S R N N S>
a a a a TRANSLATION OF FIPV N a a a a a >

490      500      510      520
*      *      *      *
AGG TCT GGT TCT CAG TCT AGA TCT GTT TCA AGA AAC AGA TCT CAA TCT
TCC AGA CCA AGA GTC AGA TCT AGA CAA AGT TCT TTG TCT AGA GTT AGA
R S G S Q S R S V S R N R S Q S>
a a a a TRANSLATION OF FIPV N a a a a a >

530      540      550      560      570
*      *      *      *      *
AGA GGA AGA CAC CAT TCC AAT ABC CAG AAT AAT AAT GTT GAG GAT ACA
TCT CCT TCT GTG GTA AGG TTA TTG GTC TTA TTA TTA CAA CTC CTA TGT
R G R H H S N N Q N N N V E D D>
a a a a TRANSLATION OF FIPV N a a a a a >

580      590      600      610      620
*      *      *      *      *
ATT GTA GCC GTG CTT GAA AAB TTA GGT GTT ACT GAC AAA CAA AGG TCA
TAA CAT CCG CAC GAA CTT TTT AAT CCA CAA TGA CTG TTT GTT TCC AGT
I V A V L E K L G V T D K Q R S>
a a a a TRANSLATION OF FIPV N a a a a a >

630      640      650      660      670
*      *      *      *      *
CGT TCT AAA OCT AGA GAA CGT AGT GAT TCC AAA OCT AGG GAC ACA ACA
GCA AGA TTT GGA TCT CTT GCA TCA CTA AGG TTT GGA TCC CTG TGT TGT
R S K P R E R S D S K P R D T D>
a a a a TRANSLATION OF FIPV N a a a a a >

680      690      700      710      720
*      *      *      *      *
CCT AAG AAT GGC AAC AAA CAC ACC TGG AAG AAA ACT GCA GGC AAG GGA
GGA TTC TTA CGG TTG TTT GTG TGG ACC TTC TTT TGA CGT CCG TTC CCT
P K N A N K H T W K K T A G K G>
a a a a TRANSLATION OF FIPV N a a a a a >

730      740      750      760
*      *      *      *
GAT GTG ACA ACT TTC TAT GGT GCT AGA AGT AGT TCA GCT AAC TTT GGT
CTA CAC TGT TGA AAG ATA CCA CGA TCT TCA TCA AGT CGA TTG AAA CCA
D V T T F Y G A R S S S A N F G>
a a a a TRANSLATION OF FIPV N a a a a a >

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This is the replacement sheet

Date: 21 OCT 1996

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770          780          790          800          810
*           *           *           *           *
GAT AGT GAT CTC GTT GGC AAT GGT AAC GCT GCC AAA TGC TAC CCT CAG
CTA TCA CTA GAG CAA CGG TTA CCA TTG CGA CGG TTT ACG ATG GGA GTC
D S D L V A N G N A A K C Y P Q>
a a a a TRANSLATION OF FIPV N a a a a a >

820          830          840          850          860
*           *           *           *           *
ATA GCT GAA TGT GTT CCA TCA GTG TCT AGC ATA ATC TTT GGC AGT CAA
TAT CGA CTT ACA CAA GGT AGT CAC AGA TCG TAT TAG AAA CCG TCA GTT
I A E C V P S V S S I I F G S Q>
a a a a TRANSLATION OF FIPV N a a a a a >

870          880          890          900          910
*           *           *           *           *
TGG TCT GCT GAA GAA GCT GGT GAT CAA GTG AAA GTC ACG CTC ACT CAC
ACC AGA CGA CTT CTT CGA CCA CTA GTT CAC TTT CAG TGC GAG TGA GNG
W S A E E A G D Q V K V T L T E>
a a a a TRANSLATION OF FIPV N a a a a a >

920          930          940          950          960
*           *           *           *           *
ACC TAC TAC CTG CCA AAG GAT GAT GGC AAA ACT AGT CAA TTC CTA GAA
TGG ATG ATG GAC GGT TTC CTA CTA CGG TTT TGA TCA GTT AAG GAT CTT
T Y Y L P K D D A K T S Q F L E>
a a a a TRANSLATION OF FIPV N a a a a a >

970          980          990          1000
*           *           *           *
CAG ATT GAC GCT TAC AAG CGA CCT TCT GAA GTG GCT AAG GAT CAG AGG
GTC TAA CTG CGA ATG TTC GCT GGA AGA CTT CAC CGA TTC CTA GTC TCC
Q I D A Y K R P S E V A K D Q R>
a a a a TRANSLATION OF FIPV N a a a a a >

1010          1020          1030          1040          1050
*           *           *           *           *
CAA AGA AGA TCC CGT TCT AAG TCT GCT GAT AAG AAG CCT GAG GAG TTG
GTT TCT TCT AGG GCA AGA TTC AGA CGA CTA TTC TTC GGA CTC CTC AAC
Q R R S R S K S A D K K P E E L>
a a a a TRANSLATION OF FIPV N a a a a a >

1060          1070          1080          1090          1100
*           *           *           *           *
TCT GTA ACT CTT GTG GAG GCA TAC ACA GAT GTG TTT GAT GAC ACA CAG
AGA CAT TGA GAA CAC CTC CGT ATG TGT CTA CAC AAA CTA CTG TGT GTC
S V T L V E A Y T D V F D D T Q>
a a a a TRANSLATION OF FIPV N a a a a a >

1110          1120          1130
*           *           *
GTT GAG ATG ATT GAT GAG GTT ACG AAC TAA
CAA CTC TAC TAA CTA CTC CAA TGC TTG ATT
V E M I D E V T N *>
a TRANSLATION OF FIPV N a a >

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This is the replacement sheet

Date: 21 OCT 1996

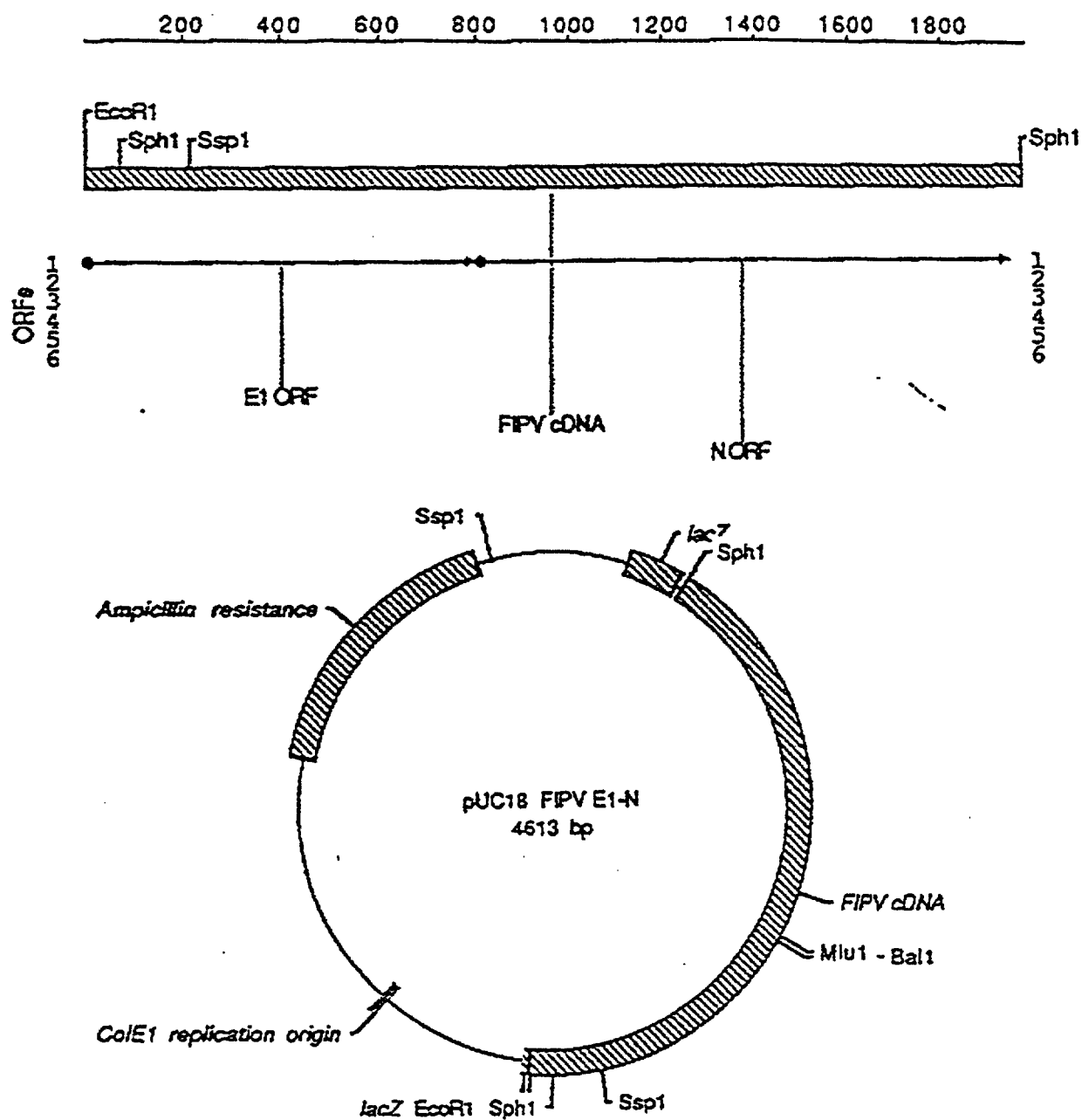


Figure 2. Plasmid pUC18:E1-N and FIPV E1 and N cDNA.

pUC18:E1-N contains a 1.9 kbp EcoRI-SspI fragment containing the FIPV E1 and N genes ligated together.

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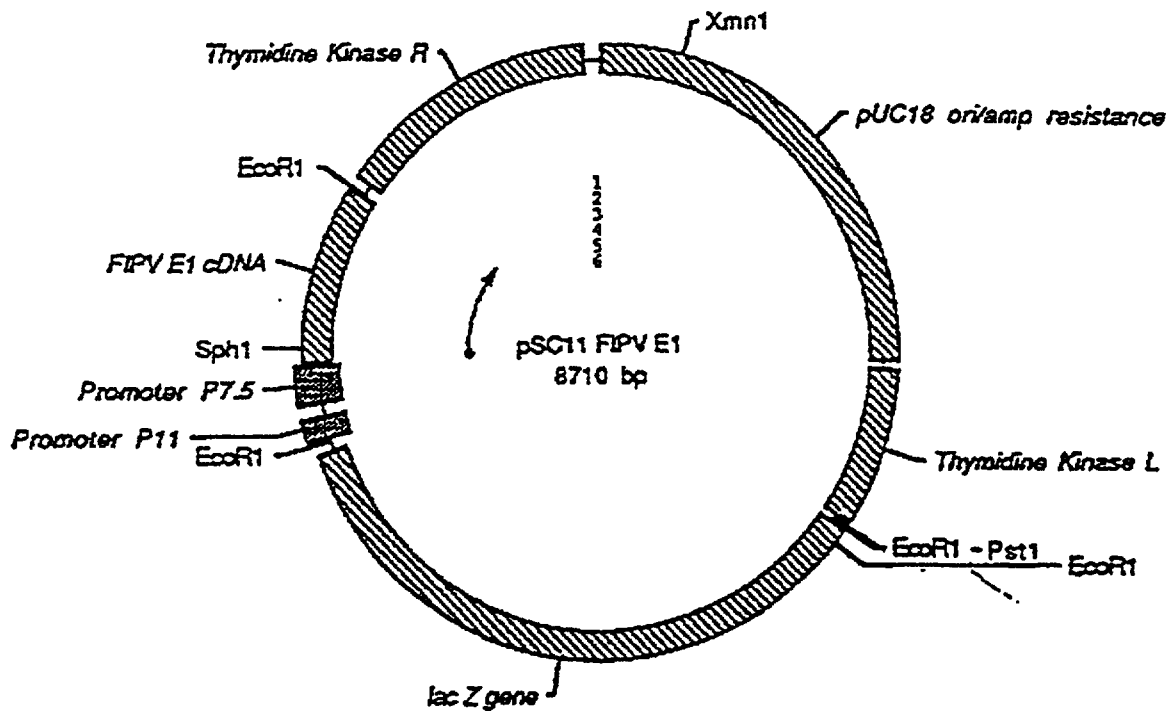


Figure 3B. pSC11 FIPV E1

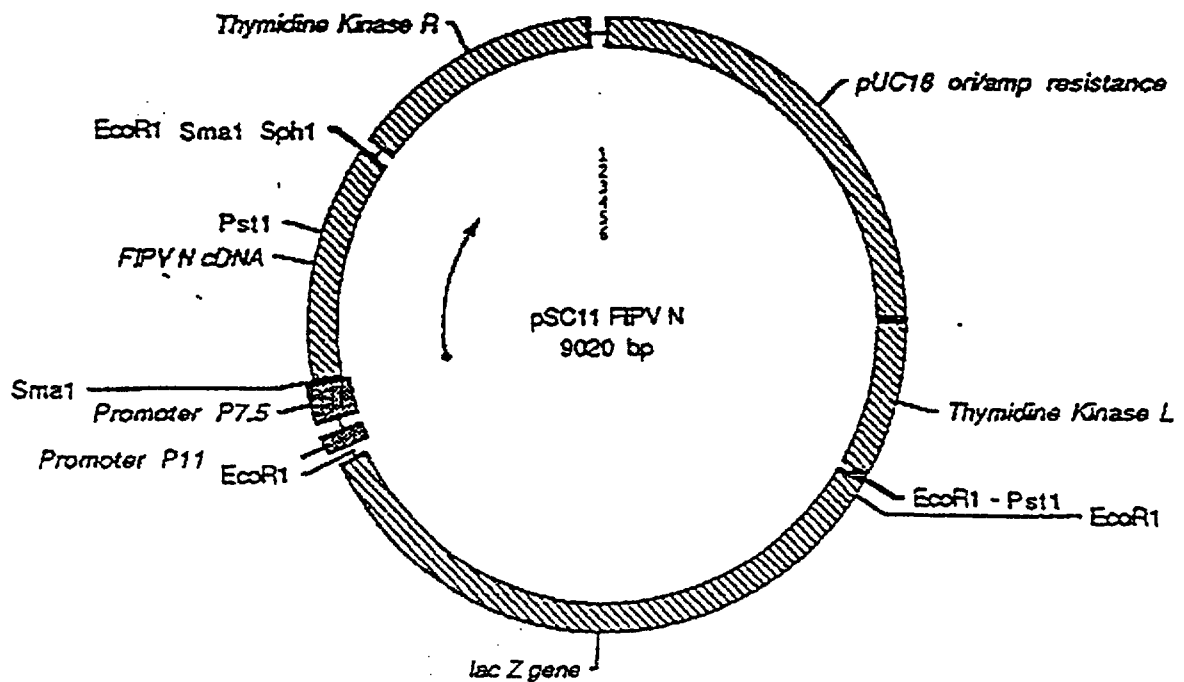


Figure 3C. pSC11 FIPV N

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FEATURES	From	To/Span	Description
DNA	1	2233	pUC18 ori/amp resistance
DNA	7377	8637	Thymidine Kinase R
DNA	6080	3030 (C)	lac Z gene
DNA	2240	3000	Thymidine Kinase L
signal	6250	6130 (C)	Promoter P11
signal	6300	6480	Promoter P7.5
DNA	6493	7329	FIPV E1 cDNA
ORF	6525	7313	1 E1 ORF

1	CGAAGGGCC	TGGTGTACG	CCATTTTCTA	TAGGTTRATG	TCATGATRAI	AATGGTTTCT
61	TAGAGGTCTAG	GTGGCATTCT	TGGGGGAAT	GTGSGGGAA	CCCTATTTTG	TTTATTTTTC
121	TAAATACATT	CAATATGTA	TCCGCTCATG	AGACAATAAC	CCTGATTAAT	GCITCATAAA
181	TATTGAAAA	GGAAGAGTAT	GAGTATTCAA	CATTTCOCTG	TGGCCCTTAT	TCCTTTTTTT
241	GCGGCATTTT	GCTTTCCTGT	TTTGTCTCAC	CCAGAAACGC	TGGTGAAGT	AAAAGTCTCT
301	GAGATCAGT	TGGGTGACG	AGTGGGTAC	ATCGAACTGG	ATCTCAGCAG	CGGTAAGTCT
361	CTTGAGAGTT	TTCGCCCGGA	AGAACGTTT	CCAATGATGA	GCACITTTAA	AGTTCGTCTA
421	TGTGGGOGGG	TATTATCOOG	TATTGACGCC	GGGCAAGAGC	AACTCGGTGG	CGGCATCTAC
481	TATCTCAGA	ATGACTTGGT	TGAGTACTCA	CCAGTCACAG	AAAAGCATCT	TACGGTGGCC
541	ATGACAGTAA	GAGATTATG	CAGTCTGCC	ATAACATGA	GTGATACAC	TGCGGCCAAC
601	TTACTCTCA	CAACATCGG	AGACCGAAG	GACCTAACCG	CTTTTTTGA	CAACATGGGG
661	GATCATGTAA	CTCGCCTGA	TGTTGGGAA	CCGGAGCTGA	ATGAAGCCT	ACCAACCGAC
721	GCGGTGACA	CCAGTCAATG	TGAGCAATG	GCAACAAAGT	TGCGCAACT	ATTAACTGGC
781	GACTACTTA	CTCAGCTTC	CCGGTACAA	TTAATAGACT	GGATGAGGC	GGTAAAGTTT
841	GCGAGACAC	TTCTGGGCTC	GGCCCTTGG	GCTGGCTGGT	TATTTGCTGA	TAAATCTGGA
901	GCGGTGAGC	GTGGGTCTCG	CGGTATCAT	GCAGCACTGG	GGCCAGTGG	TAGGCCCTCC
961	CGTATCTAG	TTATCTACAC	GAGGGGAGT	CAGGCACTA	TGGATGAGC	AAATAGACAG
1021	ATCGCTGGA	TAGGTGCTC	ACTGATTAG	CATTGGTAA	TGTCAAGCA	AGTTTCTCTA
1081	TATATACITT	AGATTGATT	AAAACITCAT	TTTAAATTTA	AAAGGATCTA	GGTGAAGATC
1141	CTTTTGTGA	ATCTCATGAC	CAAAATCCCT	TACCGTGAAT	TTTGGTTCCA	CTGAGGCTCA
1201	GACCCGCTAG	AAAAGATCAA	AGGATCTTCT	TGAGATCTTT	TTTTTCTGG	CGTATCTGCG
1261	TGCTTGCBA	CAAAAABACC	ACCGCTACCA	GCGGTGGTIT	GTTTGGCGGA	TCAGGAGCTA
1321	CCAACTCTTT	TTCCGAGGT	AACTGGCTTC	AGCAGAGGCG	AGTACCAAA	TACTGTCTTT
1381	CTAGTGTAGC	CGTAGTTAGG	CCACCTCTTC	AGACCTCTCG	TAGCAAGGCG	TACTATCTTC
1441	GCTCTGCTAA	TCTGTATACC	AGTGGCTGCT	GCCAGTGGCG	ATAAGTCTGT	TCTTACCGGG
1501	TTGACTCTAA	GACGATGAT	ACCGATGAG	GCGCAGGGGT	CGGGCTGAC	GGCGGCTTGG
1561	TGCACTACG	CCAGCTTGA	GCGAAGAAC	TACACCGAAC	TGAGTACCT	ACAGCGTGGG
1621	CATTGAGAA	GCGCAAGCT	TCCCGAAGGG	AGAAAGGGGG	ACAGGTATCC	GGTAAGCGGC
1681	AGCGTGGGA	CAGGAGAGCG	CACGAGGAG	CTTCAGGGG	GAAAGCGCTG	GTATCTTAT
1741	AGTCTGTCTG	GGTTTGGCA	CCTCTGACTT	GAGGCTGAT	TTTTTGTGAT	CTGCTCAGGG
1801	GCGCGGACC	TATGGAATA	GCGCAGAAC	GCGGCTTTT	TACGGTCTCT	GGCTTTTGG
1861	TGGCTTTTGG	CTCATATGTT	CTTCTCTGCG	TTATCCCTCG	ATTCTGTGGA	TACCGTCTAT
1921	ACCGGCTTTG	AGTGAAGCTA	TACCGCTTGC	CGCAGCGGAA	CGACCGAGCG	CAGCGATCTA
1981	GTGAGGAGG	AGGCGGAGA	GCGCCCAATA	CGCAAAACGC	CTCTCCCGCG	GCGTTGGCGG
2041	ATTCATTAA	GCGCTGGCA	CGCAGGTTT	CCCGACTGGA	AAGCGGGCAG	TAGCGCTAAC
2101	GCAATTATG	TGAGTTAGCT	CATCTATGAG	GCACCCGAGG	CTTTACCTTT	TATGCTTCGG
2161	GCTCGTATGT	TGTGTGGAT	TGTGAGGGA	TAACATTTTC	ACACAGGAA	CAGCTATGAC
2221	CATGATTAG	CCAGCTTTT	GCGATCATA	AATGGATCAC	AAACAGTATC	TCTTACGAT
2281	GTCCTTGGCA	GATGATGAT	CATTTTCTAA	GATTTTGGCT	AGTCAAGCTG	ATGAAATCTT
2341	CATTATCTGA	TATATTGCA	ATCACTCAAT	ATCTAGACTT	TCTGTATTA	TTATTGATCC
2401	AATCAAAABA	TAAATTAGAA	GCGGTGGGTC	ATTGTTATGA	ATCTCTTTCA	GAGGAATCCA
2461	GCAATTGAC	AAAAATCACA	GACTTCAAG	ATTTTAAAAA	ACTGTTTAA	AAGGTCCCTA
2521	TGTTTACAGA	TGGAGGGGTC	AAACTTAAATA	AAGGAATTTT	GTTCGCTTT	GTGATTAGTT
2581	TGATGCGATT	CAAAAAGAA	TCCTCTCTAG	CTACCAACGC	AAATAGTCTT	GTTAGATACA
2641	TAGATCTCTG	TGCAATATC	GCATTTCTTA	ACGTGRTGGA	TATATTAAAG	TGCAATAAAG
2701	TGAACATAAA	TATATTCTTT	ATTGTCTACA	TGAACGGGCG	ACATATTCTAG	TGATATATCG
2761	GCCCATGTT	TTCAAGTAAA	AGTACAGAT	TAAATTAGAG	AGTTAGAGCT	TATCAATATG
2821	CTCAATATAA	ATGCGTGAAT	ATAAAATATT	CTAACGATAA	TAGATACGGA	ACGGGACTAT
2881	GGACGGATGA	TAGAAATAT	TTTGAAGCAT	TGGAAGCAAC	TAAACTTGT	GTCTCTTGG
2941	ATTCATATAC	AGATTCTCTC	GTGATAGGTA	TGATGAAGG	ACAGTCTTT	CCAGCATTTG
3001	TTGAATTCOG	AGCTTGGGTC	CAGGTGGGG	ATCCCCCTCG	CCCGGTTATT	ATTATTTTTC
3061	ACACCGAGCC	AACTGGTAT	GATAGCAAC	GCGCTCAGC	TGATTTCCGC	CGATATCTGAC
3121	GGGCTCCAGG	AGTCTGCGC	ACCAATCCCC	ATATGGAAC	CGTCTGATTT	CAGCCATGTC

Figure 4A. Nucleotide sequence and location of various genetic elements of pSC11 FIPV E1.

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3181	CGTCTCTTCCG	CGTGCACGAC	ATGGGCGATGG	CTGGTTTCCA	TCACTTGGCG	TTGACTGTAG
3241	CGGCTGATGT	TGACTCGGA	GTCCGCGCGC	CACCTGGTGT	GGCATAATT	CAATTGCGGC
3301	GTCCGCGAC	GCAGACGGT	TTGCTGGGG	AAGAGGTACG	GGGATACAT	GTCTGACAT
3361	GGCAGATCC	AGGGTCAA	ACAGGCGCA	GTAAGGCGGT	CGGGATAGT	TTCTTGGCGC
3421	CGTATCCGA	GCCAGTTTAC	CGGCTCTGCT	ACCTGGGCA	GCTGGCAGTT	CAGGCGATC
3481	CGGCGCGAT	GCGGTGTAT	GCTGGGCACT	TCAACATCA	CGGTAATGCG	CATTGTGCA
3541	CTACCATCA	TCCGCTAGGT	TTTCCGGCTG	ATAAATAGG	TTTCCCGCTG	ATGCTGCCAC
3601	GCGTGAACCG	TGGTAATCAG	CACCGCATCA	GCAAGTGTAT	CTGCGGTGCA	CTGCAACAC
3661	GCTGCTTCGG	CCTGGTAATG	GCCGCGCGCC	TTCCAGCGTT	CGACCGAGCG	GTTAGGGTCA
3721	ATGCGGGTCC	CCTCCTTAC	GCCATGTCC	TTATCCAGCG	GTGCAAGGT	GCACTGTCC
3781	CGCAGCGCG	TCAAGAGTGG	TTTCTTATCG	CCATCCCA	TCTGTGAAG	AAGGCTGTAC
3841	TGGCGGTGA	ATTGCCACG	CTTATTAACC	AGCTCGATGC	AAAATCCAT	TTGCTGGTG
3901	GTGAGATCG	GGATGGCGTG	GGACGCGCGG	GGGAGGTCA	CCTGTAGGT	TTCCGCGTGA
3961	CGCCTCTGCT	GCCAGCGGCT	GATGTGCGCG	GCTTCTGACC	ATGCGGTCCG	TTTGGGTTC
4021	ATACAGGGA	CGTGAAGCA	GAGTTGCGCG	GCGCTCTCCG	GCTGGGGTAG	GTTGCGTAGT
4081	TCAATCACT	GTTACCTTG	TGGAGCGACA	TCCAGAGGCA	CCTCACCGCT	TGCGAGCGGC
4141	TTACCATCCA	GCGCCACCAT	CCAGTGCAGG	AGCTCGTAT	CGCTATGAGG	GATCAGGTAT
4201	TGCTTGGTCA	CTTCCATGGT	TTGCGCGGAT	AAAGGGAAT	GGAAAAGTCT	CTGCTGGTGT
4261	TTTGCTTCGG	TAGCGGCTGG	ATGCGGCGTG	CGGTCCGCA	AGCCACGACC	CTTCAATCAG
4321	AACTGGCGAT	CGTTGGGCT	ATCGCCAA	TCAAGCGGT	AAGCGGACA	CGGCTTGGCG
4381	TTTTCATCAT	ATTATATCAG	CGATGTATCC	ACCGAGTCC	AGCGAGAGCC	GCCCTGTAAA
4441	CGGGGTACT	GACCAACGC	CCTGCGATAT	TAGCGGAAC	CGCAAGACT	GTTACCATC
4501	GCGTGGGCT	ATTGCAAG	GATCAGCGCG	CGGTCTCTC	CAGGTAGCGA	AAGCATTTT
4561	TTGATGGAC	ATTGCGGAC	AGCGGGGAG	GCTGGGTCT	CATCCAGCG	CGGATACATC
4621	GGGATATAA	TATGGTGGC	CGTGGTGTGG	GCTGCGCGCG	CCTCATACTG	CACCGGGCGG
4681	GAGGATCCA	CAGTTTGTAT	CCAGCGATAC	AGCGCGGTCT	GATTAGCGCC	GATGGCTGAT
4741	TCAATCCCA	GCGACAGAT	GATCCTCTC	GCGTGAATAC	GATCGCGCTG	CACCATTCGC
4801	GTTAGCGCT	CGCTATCGC	CGGTAGCGAG	CGCGATCAT	CGGTAGAGG	ATTGATGGC
4861	ACCATGCGT	GCGTTCAAT	ATTGGCTTCA	TCCACCAT	ACAGCGGTA	GCGGTGCGAC
4921	AGGTGTAC	ACAGCGGATG	GTTGCGATTA	TCCGACAGC	GACCGGCTT	AAAGTGTTC
4981	TGCTTCACTA	GCGAGATATC	CTGCAACATC	GCTGCTCAT	CCATGACCTG	ACCATGCGA
5041	GATGTATCT	CGTGGCGGT	AACGCTTGA	ATCAGCAAG	GCTTGGCGGT	CAGCAGCGA
5101	AGACCATTT	CATCCGCGC	CTCGCGGAA	CGCATCTGC	AGGCTTCTGC	TTCAATCAGC
5161	GTGCGGTGG	CGGTGTGCG	TTCAACAC	GCAAGATAG	GATCGGGAT	TTCCGCGCTC
5221	CACAGTTTCG	GGTTTTCGAC	CTTGAAGCT	AGTGTGAGCG	GATGGGATA	ACCATCCGCG
5281	TCAATGATA	TTTACCGCG	GAAAGCGCG	GTGCGGTGG	CGACCTGGGT	TTCACTTGC
5341	CATAAGGAA	CTGTAACCG	TAGGTAGTCA	CGCAACTGC	CGCATCTG	AACTTACGCG
5401	TCCAGTACAG	CGCGGCTGAA	ATCATCATTA	AAGCGAGTGG	CACATGGA	ATCGCTGATT
5461	TGTGTAGTCC	GTTATGCGAG	CACGAGAGCG	TACCGGAAA	TGCGGCTCAT	CCGCGACATA
5521	TCTGTATCT	CCAGATAACT	GCGGTCTCTC	CACCGGAGCA	CCATCACCGC	GAGGCGGTTT
5581	TCTGCGCGCG	GTAATAATGC	GCTCAGGTCA	AATTCAGAGC	GCAAAAGCAT	GTCCGCGCG
5641	TACCGGACCG	AGCGCGGCT	GACCCACAGA	TGAAGCGCG	AGTAAAGCG	ATCAAAAATA
5701	ATTGCGGTCT	GCGCTTCTCG	TAGCCAGCTT	TCTCAACAT	TAAATGTGAG	CGAGTACAA
5761	CGGTCGGAT	TCTCGGTGGG	AACCAAGCGG	GGAATGCGG	TATGGGATA	GGTACGTTG
5821	GTTGAGTGG	GCGCATCGTA	ACCGTGCACT	TCCAGTGTG	AGCGGACGAC	GACAGTATCG
5881	GCTCAGGAA	GATCGCACTC	CAGCCAGCTT	TCCGGCACCG	CCTCTGGTGC	CGGAACCGAG
5941	GCAAGCGCG	ATTGCGGCT	CAGGCTGGCG	AACCTGTTGG	AAGGCGGCTC	GGTGCGGCGC
6001	TCTTGGCTAT	TACCGGAGCT	GCGGAAAGGG	GGAATGTGCG	CAGGCGGAT	AGGTTGGGTA
6061	ACCGCAGGGT	TTTCCAGTCC	ACGACGTTGT	AAAACGAGCG	GATCCCTCGA	GGAATTCAT
6121	TATAGCATAG	AAAAAACA	AATGAAATTC	TACTATATT	TTCAATACAT	ATATTCTAAA
6181	TATGAAGTG	GATGTTGTGA	CTAGCGTAGC	ATCGCTTCTA	GACATATCT	ATATAGTAA
6241	ACCATATCTC	AGACTACGA	AACGTATACA	ATCTCTTATC	ATGCGGTA	TGTTCTCGAT
6301	GTCGATAGC	CATATGCGCG	TAGTTGCGAT	ATACATTAAC	TGATCACTTA	TTCCAAACCC
6361	ACCGGCTTTT	TATAGTATG	TTTTCACCA	TAAATATTA	ATCATATAT	TATTTCTCG
6421	TAAAGTACA	AAATATATTC	TATTTATTTG	CACGGTAAAG	AAGTAGATC	ATPAAGACAA
6481	GTGACGGATC	CCATTCGCG	CATTTTGGT	TGAACATAA	CRAATGAAG	TACATTTTGC
6541	TATATCTGCG	GTGCATAATT	GCTGCGTTT	ATGGTGAAG	CATCTGTGCC	ATGCAAGACA
6601	GTTGCTTGA	GTGATTAAT	GCCACAAAT	CAAGATGTA	AACCGGCTT	GACGTTGGG
6661	ATCTTATTTG	GCACTTGTCT	AACGTGAATC	TGAGCTGGTC	TGATATTTG	ATTGTTTTTA
6721	TAAAGTGT	ACAAATGCGC	AGCCACAAAT	TGAGCTGGCT	CGTTTATGCG	ATTAAGATGC
6781	TGATCATGTG	GCAATATATG	CGATTTGCTC	TAGCGCTTAC	GATTTTAT	GCACTCTCTG
6841	AGTACCAAGT	TTCCAGTAT	GTAATGTTCC	GCTTATAGT	TGCGGTGCA	GTTGTACGT
6901	TTGCACTTGG	GATGATGAT	TTTGTGAGAT	CGTTTACGT	AAATAGAGA	ACCAATCAT

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6961 GGTGGTCITT TAACTCTGAG ACTAATGCAA TTCCTTGTGT TAATGCATTG GGTAGAGATT
7021 ATGTGCTTCC CTTAGATGGT ACTOCTACAG GTGTBACCTT TACTCTACTT TCAGGAATC
7081 TATATGCTGA AGGTTTCBAA ATGGCTGGTG GTTTAACCCT CGAGCAITTG CCTAATACG
7141 TCATGATTGC TACAOCTAGT AGAACCATCG TTTATACATT AGTTGGAAA CATTAAACG
7201 CAACTACTGC CACAGGATGG GCTTACTAOG TAAATCTAA AGCTGGTGT TACTCACAG
7261 AAGCACTAC TGACBATTG AGTGAACATG AAAATTATT ACATATGGTG TAACTBACT
7321 TTCAAATGGG GGAATTCGT GAGGTATGG CBAACGAGG ABAATTAGT TATAGTAGCC
7381 GCACTOGATG GGCATTTC ACGTAAACOG TTTABATAA TTTTGAATCT TATTOCTTA
7441 TCTGAATGG TGGTAAACT AACTGCTGTG TGTATGAAT GCITTAAGGA GGCITTOCTT
7501 TCTAAGCAT TGGGTGAGGA AACCGGATA GAATTAATG GAGGTAATGA TATGATCAA
7561 TCGGTGTGTA GAAAGTGTA CATCGACTCA TAAATATTATA TTTTATCTCT AAAAATCAA
7621 AAATAACAT TGATTAAAT TTAATATAAT ACTTAAABAT GGATGTTGTG TCGTATGTA
7681 AACCGTTTAT GTATTTTGAG GAAATGTATA ATGAGTTAGA TTACGACCA GAAAGTGCAA
7741 ATGAGGTGCG AAAAABACTG CGTATCAAG GCAGTTAAA ACCTTACTA GGAATATTAT
7801 TTTTCTTAG TAAGTTACAG CGCACGGTA TATTBGATGG TGCCACCGTA GTGTATATAG
7861 GATCTGCTCC CGGTACCAT ATACGTTAT TCGAGATCA TTTCTATAT TTAGGATGA
7921 TCATCAATG GATGCTAAT GACGGGOGC ATCATGATC TATTTTAAAT GGTGOGTG
7981 ATGTACTCT AGTGACTOGG TTGTTGATG AGGAATATCT ACGATCATC AAAAACHAC
8041 TGCATCCTC TAACTTAT TTAATTTCTG ATGTGAGTC CAAACGAGA GGAATGAAC
8101 CTAGTACGGC GGATTACTA AGTAAATAG CTCTACAAA TGTATGATT AGTATTTTAA
8161 ACCCGTGGC GTCTAGTCTT AATGGAGAT GOCOGTTTC AGATCAATG ATCAGGACT
8221 TTTATATCC ACAOGGTAAT AAAATGTTAC ABOCTTTTG TOCTCATAT TCAGGGOGT
8281 CGTTTTACAA CGTGTGACT GGGAAACOC TGGOGTTACC CAACTAATC GOCITGAGC
8341 ACATCOOCT TTGOCAGCT GOGTAAATAG CGAAGAGGOC CGCAOCATC GOCITCOCA
8401 ACAGTTGOC AGOCGATG GOGAATGGG OCTGATGOG TATTTCTCT TTAOCATCT
8461 GTGOGTATT TCACACOGCA TATGGTGAC TCTGATACC ATCTGCTG ATGOCGATA
8521 GTTAGOCAG TACCTOOGC TATOGTTAG TGACTGGTC ATGGTGGC CCGACACOC
8581 GCGAACACOC GCTGACGOC CCGAOGGCT TTGCTGCTC CCGCATOCG CTACAGACA
8641 AGCTGTGAC GTCTOOGGA GCTGATGTC TCAGAGGTT TCACCGTAT CAOCGAOC
8701 CGGAGGCG

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FEATURES	From	To/Span	Description
DNA	1	2233	pOC18 ori/amp resistance
DNA	7687	8947	Thymidine Kinase R
DNA	6080	3030 (C)	lac Z gene
DNA	2240	3000	Thymidine Kinase L
signal	6250	6130 (C)	Promoter P11
signal	6300	6480	Promoter P7.5
DNA	6496	7636	FIPV N cDNA
ORF	6496	7629	1 N ORF

1	CGAAGGGGOC	TGTGTATACG	CCATTTTITA	TAGGTTAATG	TCATGTAAT	AATGGTTTCT
61	TACACGTCTAG	GTGGCACTTT	TGGGGGAAAT	GTGGGGGAA	CCCTATTG	TTTATTTTTC
121	TAAATACATT	CAATATGTA	TCCGTCATG	AGACAATAC	CCGTATAAT	GCCTCAATBA
181	TATTGAAAAA	GGAAGATAT	GATATTCAA	CATTTCGGT	TGCGCTTAT	TCCCTTTTTT
241	GCGGCATTTT	GCCTTCCTGT	TTTGTCTAC	CCAGAACGC	TGGTGAAGT	AAAGCTTGCT
301	GAAGATCAGT	TGGGTGCAAG	AGTGGGTTAC	ATCGAACGG	ATCTCAACG	CGGTAGATC
361	CITGAGAGTT	TTGCCCCGA	AGAAGTTTT	CCATGTATG	GCACTTTTAA	AGTTCTGCTA
421	TGTGGCGGG	TATTATCCCG	TATTGACGCC	GGCAGAGC	AACCTGGTC	CCGCTATAC
481	TATTCTCAGA	ATGACTTGGT	TGAGTACTCA	CCAGTCAAG	AAAGCATCT	TACCGTTGGC
541	ATGACAGTAA	GAGATTATG	CAGTCTGCG	ATACCATGA	GTGATACAC	TGCGGCCAC
601	TTACTTCTGA	CAACGATCGG	AGGACCGAG	GAGCTAACCG	CTTTTITGCA	CAACATGGGG
661	GATCATGTAA	CTCGCCTTGA	TGTTTGGGA	CCGGAGCTGA	ATGAAGCCAT	ACCAAGACG
721	GACCGGTACA	CCACGATGCG	TGTAGCARTG	GCAACAAGT	TGCGCAACT	ATTACTGGG
781	GAACTACTTA	CTCTAGCTTC	CCGGCAACA	TTAATAGACT	GGATGGAGC	GGTAAAGTT
841	GCAGGACAC	TTCTGCGCTC	GGCCCTCCCG	CTTGGCTGGT	TTATTGCTGA	TAAATCTGGA
901	GCGGGTGAGC	GTGGGTCTCG	CGGTATCAAT	GCAACACTGG	GGCCAGATG	TAAGCCCTCC
961	CGTATCGTAG	TTATCTACAC	GACGGGAGT	CAGGCAACTA	TGGTGAACG	AAATAGACAG
1021	ATCGCTGAGA	TAGGTGCCCTC	ACTGATTBAG	CATTGGTAC	TGTACAGCA	AGTTTACTCA
1081	TATATACATT	AGATTGATT	AAACTCTCT	TTTTAATTA	AAAGGATCTA	GGTGAAGATC
1141	CTTTTTGATA	ATCTCATGAC	CAAAATCCCT	TACGTGAGT	TTTGGTTCCA	CTGAGCGTCA
1201	CGTCCGCTAG	AAAAGATCAA	AGGATCTTCT	TGAGATCCTT	TTTTCTGCG	CGTATCTGCG
1261	TGCTTGCAAA	CAAAAAAACC	ACCGCTTCCA	GCGGTGGTTT	GTGTGCGGA	TCAAGAGCTA
1321	CCAACTCTTT	TTCCGAGGCT	AACTGGCTTC	AGCAGGCGC	AGTACCAAA	TACTGTCCCT
1381	CTAGTGTAGC	CTAGTGTAGG	CCACCACTTC	ANGACTCTG	TAGCACCGCC	TACTATCCCT
1441	CTCTGCTTAA	TCTGTATAC	AGTGGCTGCT	GCCAGTGGG	ATAAGTCTG	TCCTTACCGG
1501	TTGACTCTAA	GACATAGATT	ACCGGATAG	GCGCAGCGG	CGGCTGAACT	GGGGGGTTCG
1561	TGCACACACG	CCAGCTTGGG	GCGAAGACC	TACACCGAC	TGAGATACCT	ACAGCGTGG
1621	CATTGAGAAA	GCGCCACGGT	TCCCGAGGG	AGAAGGGGG	ACAGGTATCC	GGTGAAGCGG
1681	AGGGTCGGA	CAGGAGAGCG	CAAGGAGAG	CTTCCAGGG	GAAACGCTG	GTATCTTAT
1741	AGTCTGCTCG	GGTTTGGCCA	CTCTGACTT	GAGCGTCAAT	TTTTGTGATG	CTCGTACGGG
1801	GGGCGGAGCC	TATGGA AAAA	CGCCAGCAAC	GCGGCTTTT	TACGGTTCCT	GGCCTTTTGC
1861	TGGCTTTTTC	CTCAGATGTT	CTTCTCTGG	TATCCCTCG	ATCTGTGGA	TACCGTATT
1921	ACCGCCCTTG	AGTGAAGCTGA	TACCGCTCG	CCAGCGGGA	CGACCGAGCG	CAGCGAGTCA
1981	GTGAGCGAGG	AAGCGGAGA	GCGCCAACTA	CCCAACCGC	CTCTCCCGC	GCGTGGCGG
2041	ATTCATTAT	GCAGCTGGCA	GACAGGTTT	CCGACTGCA	AAGCGGGGAG	TEAGCGCAAC
2101	GCAATTAAAG	TGAGTTAGCT	CATCTATTAG	GCAACCCAGG	CTTACACAT	TATGCTTCCG
2161	GCTGCTATGT	TGTGTGGAAT	TGTGAGCGGA	TACCAATTC	ACACAGGAA	CAGCTATGAC
2221	CATGATTACG	CCAAGCTTTT	GCGATCAATA	AATGGATCAC	ABCCAGTATC	TCTTAAACAT
2281	GTCTCTGCA	GATGATGATT	CATTTTTAA	GTATTTGGCT	AGTCAAGATG	ATGAAATCTT
2341	CATTATCTGA	TATATTGCAA	ATCACTCAAT	ATCTAGACTT	TCGTATTATA	TTATTGATCC
2401	AATCAAAAAA	TAAATTAGAA	GCGGTGGGTC	ATTGTTATGA	ATCTCTTTCA	GAGGATACAA
2461	GACAAATTGAC	AAAATTCACT	GACTTTCAAG	ATTTTAAAAA	ACTGTTTAA	AAGGTCCCTA
2521	TTGTTACAGA	TGGAGGGGTC	AAACTTAATA	AAGGATATT	GTTCGACTTT	GTGATTAGTT
2581	TGATGCGATT	CAAAAAAGAA	TCTCTCTGAG	CTACCCCGC	AATAGATCCT	GTAGATACAA
2641	TAGATCCTCG	TCCCAATATC	GCATTTCTTA	ACGTGATGGA	TATATTAAAG	TGCAATTAAG
2701	TGAACAATAA	TTAATCTTTT	ATTGTCTCA	TGACGGCGG	ACATATTCCG	TTGATATATG
2761	GCCCCATGTT	TTCAAGTAAA	AGTACAGAT	TAAATTAGAG	AGTTAGACGT	TATCAATAG
2821	CTCAATATAA	ATGCGTGACT	ATAAATATT	CTAACGATBA	TAGATACGGA	ACGGGACTAT
2881	GGACGCATGA	TAAGATAAT	TTTGAAGCAT	TGGAGCAAC	TAACTATGT	GATCTCTTGG
2941	AATCAATTAC	AGATTCTCTC	GTGATAGGTA	TCGATGAAG	ACAGTTCTTT	CCAGACTTGG
3001	TTGAATTCGG	AGCTTGGCTG	CAGGTGGGG	ATCCCGCTG	CCCGTTTATT	ATTATTTTGG
3061	ACACCAAGAC	AACTGGTAA	GGTAGCGAAC	GCGCTCAGC	TGAATTCGCG	CGATACAGAC
3121	GGGCTCCAGG	AGTCTGCGCC	ACCAATCCCG	ATATGGAAC	CGTGGATATT	CAGCCATGTC

Figure 4B. Nucleotide sequence and location of various genetic elements of pSC11 FIPV N.

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3181	CGTCTCTCCG	CGTGGGCGAG	ATGGCGGTCG	CTGGTTTCCA	TCAGTTGCTG	TTGACGGTAG
3241	CGGCTGATGT	TGACTGGA	GTCCGCGCGC	CACGCTGCTG	GGCCTAAT	CAATTCGCGC
3301	GTCCCGCAGC	GCGACCGT	TTCCGTCGGG	AGGACGTACG	GGGTATACAT	GTCTGACAT
3361	GGCGATGCC	AGCGGTCAA	ACAGCGCGCA	GTAGCGCGGT	CGGATAGTT	TTCTTCGGGC
3421	CCTAATCCGA	GCCAGTTTAC	CCGCTCTGCT	ACCGCGCGCA	GCTGGCGGTT	CAGGCGCATC
3481	CGCGCGGAT	GCGGTGTATC	GCTCGGCT	TCAACATCAA	CGGATATCGC	CATTTGACCA
3541	CATACCATCA	TCCGCTAGGT	TTTCCGCGTG	ATAAATAGG	TTTCCCGCTG	ATGCTGCCAC
3601	GCGTGCAGCG	TGGTATTCAG	CACCGCATCA	GCAAGTGTAT	CTGCGGTGCA	CTGCACACAC
3661	GCTGCTTCGG	CCTGGTAAATG	GCCCGCGCGC	TTCCAGCGTT	CGACCGAGGC	GTTAGGGTCA
3721	ATGCGGGTGG	CTTCGCTTAC	GCCATATGTC	TTATCCAGCG	GTCACCGGTT	GAACGTATCG
3781	CGCGCGCGCG	TGAGCGCTTG	TTTCTTATCG	CGATCCACCA	TCTGTGAAG	AAAGCGCTAC
3841	TGGCGGTBA	ATTGCCACAG	CTTATACCC	AGCTCGATGC	AAAAATCCAT	TTCGCTGGTG
3901	GTGAGATGGG	GGATGCGGTG	GGACGCGCGC	GGAGCGGTCA	CACGTAGGTT	TTCCGCGCA
3961	CGCGCTGCT	GCCAGGCGCT	GATGTGCGCG	GCTTCGACCC	ATGCGGTCGC	GTTCCGTTGC
4021	ACTACCGGTA	CCTGAGGCA	GAGTTGCGCG	GCGCTCTCG	GCTGCGGTAG	TTCAGGCGGT
4081	TCAATCAACT	GTTAACCTG	TGGAGCGACA	TCCAGGCGCA	CTTACCGGTT	TCCGCGCGCG
4141	TTCCATCCCA	GCGCCACCAT	CCAGTGCAGG	AGCTCGTTAT	CGCTATGACG	GAACGCGTAT
4201	TGCGCTGCTA	CTTCGATGCT	TTGCGCGGTT	AAACGCAACT	GGAATAACCTG	CCTGCTGCTG
4261	TTTGTCTCG	TGAGCGCTGG	ATGCGCGGTG	CGGTGCGCA	AGACCGAGCC	GTTCAATACAG
4321	AACTGGCGAT	CGTTCGGGCT	ATCGGCACAA	TCCCGCGGCT	AAGCGCGCA	CGGCTTGGCG
4381	TTTTCTATCT	ATTTAATCAG	CGACGCTCC	ACCGAGTCC	AGACGAGGCG	GGCTGTAAA
4441	CGGGGATCT	GACGAAACG	CTCGCATAT	TTAGCGAAAC	CGCCAGACT	GTTACCGCTC
4501	GCGTGGCGGT	ATTGCGCAAG	GATCAGCGGG	CGGCTCTCTC	CAGGTAGCGA	AGGCGATTTT
4561	TGATGGGAC	ATTTCGGGAC	AGCGCGGAG	GGCTGGCTTT	CATCCAGCG	CGGCTATCTC
4621	GGCGAATAA	TATCGGTGGC	CGTGGTGGG	GCTCGCGCGC	CTTCACTCTG	CAGCGGCGCG
4681	GAAGGATCGA	CAGTTTGTAT	CCAGCGATAC	AGCGCGTGGT	GATTAGCGCC	GTGGCGCTAT
4741	TCAATCCCA	GCGCCAGAT	GATCAGCTC	GGGTGATAC	GATCGCGCTG	CACCATTCGC
4801	GTTACCGGTT	CGCTCATCC	CGGTAGCGG	CGCGGATCAT	CGGTGACG	ATTGATGGC
4861	AGACCATTTT	GGGTCTCAT	ATTGGCTTCA	TCCACCATAT	ACAGCGCGTA	GGGTTCGCAC
4921	AGCGTGTACC	ACAGCGGATG	GTTCCGATAA	TGCGACAGC	GCGCGCGGTT	AAAGTTGCTC
4981	TGCTTCATCA	GCGGATATC	CTGCACCATC	GCTGCTCAT	CCATGACCTG	ACCATGCGA
5041	GGATGATGCT	CGTGCAGGTT	AAAGCGTCCA	ATCAGTAAAG	GCTTCCGCTT	CAGCAGGACG
5101	AGACCATTTT	CAATCCGAC	CTCGCGGAAA	CGGACATCGC	AGGCTTCTCG	TTCAATCAGC
5161	GTCGCGTGGG	CGGTGTGCG	TTCAACCAAC	GCAAGTACAG	GATTGGGAT	TTCCGCGCTC
5221	CACGATTTGG	GGTTTTCGAC	CTTGCAGCT	AGTGTGAGCG	GATCGGCTAT	ACCAACAGCC
5281	TCAATCGTAA	TTTCCCGGCT	GAAAGTGGG	GTCGCGCTGG	CGACCTGCTG	TTCCCGCTGC
5341	CATAAGGAA	CTGTACCGG	TAGGTAGTCA	CGCACTCGC	CGCATCTCTG	AACTTCAGCC
5401	TCCAGTACAG	CGCGGCTGAA	ATCATCATTA	AAAGGATGGG	CACATGGA	ATCGCTGAT
5461	TGTGTAGTGG	GTTTATGCGG	CACCGGAGCG	TCCGGAATA	TGCGGCTCAT	CGGCGACATA
5521	TCTGTCTCTT	CCGATATACT	GGGCTCTCTC	CACCGTACCA	CCATCACCGC	GAGGCGGTTT
5581	TCTCGCGGCG	GTAATAATGC	GCTCAGGTCA	AACTCAGAG	GCAACGACT	GTCTGGCGCG
5641	TACCGGACCC	AGCGCGGTTT	GCAACACAGA	TGAACCGCGG	AGTTAACGCT	ATCAAAATA
5701	ATTCCGGCTCT	GGGCTTCCG	TAGCCAGCTT	TCTCAACAT	TBAATGTGAG	CGAGTACCAA
5761	CCCGTGGGAT	TCTCCGTTGG	AACCAACGCG	GGATGACCG	TBATGGGATA	GGTACGTTG
5821	GTTGATGATG	GCGCATGCTA	ACCGTGCATC	TGCCAGTTTG	AGGGGAGCGC	GACGATATCG
5881	GCTTCAGGAA	GATCGCACTC	CAGCGAGCTT	TCCGCGACCG	CTTCTGGTGC	CGGAAACCG
5941	GCAAGCGGCG	ATTCCGCAAT	CAGGCTGCGC	AACTGTTGGG	AAGGGCGATC	GGTGGCGGCG
6001	TCTTCGCTAT	TCCGCGAGCT	GCGGAAAGCG	GGATGTGCTG	CAAGCGGAT	AAGTTGGGTA
6061	ACGCGAGGCT	TTTCCAGTTC	ACGAGTTTGT	AAAACGAGCG	GATCCCTCGA	GGATTCAT
6121	TATAGCTTAG	AAAAAACA	AATGAATTC	TACTATATT	TTACATACAT	ATATTCGAA
6181	TATGAAGTGG	GTTGATTGTA	CTAGCTAGC	ATCGCTTCTA	GACATATCT	ATATAGTAT
6241	ACCAATCTC	AAGACTACGA	AATGTATACA	ATCTCTTATC	ATGTGGTAA	TGTTCTCGAT
6301	GTCGATATGC	CATATGCGGG	TAGTTGCGAT	ATACATTAAC	TGATCACTAA	TTCCAAACCC
6361	ACCGGCTTTT	TATAGTAAAT	TTTTCACCGA	TAAATATTA	ATACATATAT	TAAATTCCTG
6421	TAAAGTAGA	AAATATATTC	TAAATTTATG	CACGGTACGG	AAGTAGAATC	ATAAAGACA
6481	GTCACGGATC	CGGGATGGC	CACACAGGGA	CAACGCTCA	ACTGGGAGAG	TGACCTTCC
6541	AAAAGACGTG	GTCGTTCTAA	CTTCGCTGGT	CGGAGGATA	ATGTATATAC	TTTGTCTATC
6601	TACACCCCA	TTCGCTCGA	ACAAGGATCT	AAATTTTGA	ATTATGTGTC	GAGAGACCTT
6661	GTTCCCAAG	GATAGGTAA	TAGGATCAA	CRAATTTGTT	ATTGGATGAG	ACAGTTGCTG
6721	TATCGTATCTG	TAAAGGCGCA	GCGTACGGA	CTCGCTGAGA	GCTGCTCTTT	TTACTCTTA
6781	GCTACAGGAC	CTCTGCTGA	TGCTAAATTC	AAAGCAAGA	TTGATGAGT	CTTCTCGGTT
6841	GCAAGGGATG	GTCGCTGGA	CAGGCGGCA	ACGTTTGGCA	CTCGTGGAC	CATATACGGA
6901	TCCAAACCCAC	TGAGGTTTGA	TGCTAGATA	CGGACAGT	TTCACTTGA	AGTGAACCGT

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6961 TCTAGGAACA ATTCAAGGTC TGGTTCTCAG TCTAGATCIG TTTCAAGRAA CAGATCTCAA
 7021 TCTAGAGGAA GACACCATTC CAATAAOCAG AATAATAATG TTGAGGATAC AATTGTAGCC
 7081 GTGCTTGAAA AATTEAGGTGT TACTGACAAA CAABGGTCAC GTTCTAAAC TAGAGAAAGT
 7141 AGTGATTCCA ABOCTAGGGA CACAACAOCCT AAGAATGCOA ACAAACACAC CTGGAAGAAA
 7201 ACTGCAGGCA AGGGAGATGT GACAACTTTC TATGGTGCTA GAAGTAGTTC AGCTAATTT
 7261 GGTGATAGTG ATCTCGTTGC CAATGGTAAC GCTGCCAAAT GCTACCCCTCA GATAGCTGAA
 7321 TGTGTTCCAT CAGTGTCTAG CATATCTTT GGCAGTCAAT GGTCTGCTGA AGAAGCTGGT
 7381 GATCAAGTGA AAGTCACGCT CACTCACAC TACTACCTGC CAAAGGATGA TGCCAAABCT
 7441 AGTCAATTOC TAGAACAGAT TGACGCTTAC AAGCGAOCCT CTGAAGTGGC TAAGGATCAG
 7501 AGGCAAGAAA GATCCCGTTC TAAGTCTGCT GATAAGAAGC CTGAGGAGTT GTCTGTAACT
 7561 CTGTGGGAGG CATACACAGA TGTGTTTGAT GACACACAGG TTGAGATGAT TGATGAGGTT
 7621 ACGAACTAAA CGCATGCCOG GGAATTCCTGT GAGOGTATGG CAAACGAGG AAAAATTAGT
 7681 TATAGTAGCC GCACCTCGATG GGACATTTCA ACGTAACCG TTTAATAATA TTTTGAATCT
 7741 TATTCATTA TCTGAAATGG TGGTAAAAC AACTGCTGTG TGTATGAAAT GCTTTAAGCA
 7801 GGCCTCCCTT TCTAAACGAT TGGGTGAGGA AACCGAGATA GAAATATAG GAGGTAAATGA
 7861 TATGTATCAA TCGGTGTGTA GAAAGTGTIA CATCGACTCA TAATATTATA TTTTATATCT
 7921 AAABAACTAA AATAAACAT TGATTAAAT TTATATAAT ACTTAAABAT GGATGTGTG
 7981 TCGTTAGATA AACCGTTTAT GTATTTTGAG GAATTGATA ATGAGTTAGA TTACGAAOCC
 8041 GAAAGTGCAA ATGAGGTGCG AAAAABACTG CGTATCBAAG GACAGTTAA ACTATTAATA
 8101 GGTGATTTAT TTTTCTTAG TAAGTTACAG CGACACGGTA TATTAGATGG TGCCACOGTA
 8161 GTGTATATAG GATCTGCTOC CGGTACACAT ATAGTTATT TGAGAGTCA TTTCTATAAT
 8221 TTGGGAGTGA TCATCAAATG GATGCTAATT GACGGCOCOC ATCATGATCC TATTTTAAAT
 8281 GGAATGCGTG ATGTGACTCT AGTGACTCGG TTCGTTGATG AGGAATATCT ACGATCCATC
 8341 AAABAAACAC TGCACTCTTC TARGATTATT TTAATTTCTG ATGTGAGTTC CAACCGAGGA
 8401 GGAATGAAC CTAGTAOGGC GGATTTACTA AGTAATTACG CTCTACABAA TGTATGATT
 8461 AGTATTTTAA ACCCGGTGGC GTCTAGTCTT AAATGGAGAT GCGCGTTTC AGATCAATGG
 8521 ATCAAGGACT TTATATATCC ACACGGTAAAT AAATGTTAC AACCTTTTGC TCCTTCATAT
 8581 TCAGGGCCGT CGTTTACAA CGTCTGTACT GGGRAAACC TGSCGTTAOC CAECTTATC
 8641 GGCCTGCAGC ACATCCOCT TTGOCAGCT GGCGTAATAG CGAAGAGGOC CGCAOCGATC
 8701 GCGCTTCCCA ACAGTTGOGC AGOCTGAATG GCGAATGGOG CCTGATGGCG TATTTTCTCT
 8761 TTACGCATCT GTGOGGATTT TCACACOGCA TATGGTGAC TCTCAGTACC ATCTGCTCTG
 8821 ATGCGGCTAT GTTAAGOCAG TCACTCTCGC TATOGCTACG TGACTGGGTC ATGGCTGOGC
 8881 CCGGACACCC GOCACACCC GCTGACGGC CCTGBOGGC TTGTCTGCTC CCGGCATCCG
 8941 CTTACAGACA AGCTGTGACC GTCTCOGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAAT
 9001 CACCGAARCG CGCGAGGCAG

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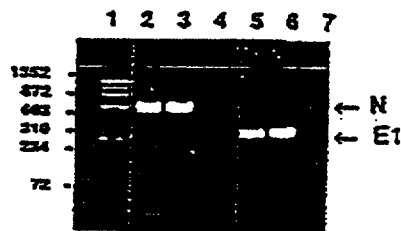


Figure 5. PCR analysis of rRPV-FIPV N and E1 viral-cell lysates.

Lane 1, Base pair DNA markers; Lane 2, pSC11 FIPV N (FIPV N positive control); Lane 3, rRPV-FIPV N; Lane 4, wild-type raccoon pox virus-cell lysates with FIPV N primers (negative control); Lane 5, pSC11 FIPV E1 (FIPV E1 positive control); Lane 6, rRPV-FIPV E1; Lane 7, wild-type raccoon pox virus-cell lysates with FIPV E1 primers (negative control).

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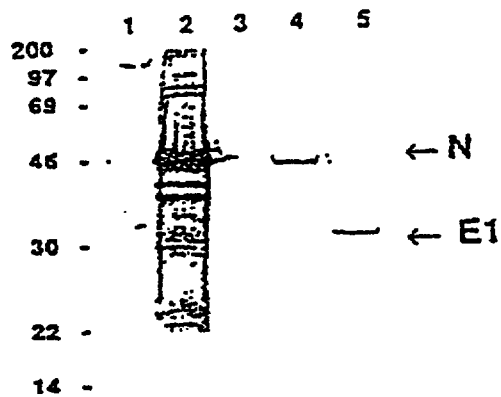


Figure 6. Western blot analysis of rRPV-FIPV N and E1 viral-cell lysates.

Lane 1, molecular weight markers (kDa); Lane 2, purified FIPV antigen (positive control); Lane 3, wild-type raccoon pox virus-cell lysate (negative control); Lane 4, rRPV-FIPV N; Lane 5, rRPV-FIPV E1.

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

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

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 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 (also known as the M) (transmembrane) protein, and the N (nucleocapsid) protein. Venema et al., Virology 181:327-335, 1991 and Dale, et al., 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., 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

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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. The terms "E1" and "M" as used herein, in the context of the transmembrane protein, refer to the same transmembrane protein.

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.

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In accordance with a first aspect of the present invention there is provided
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).

In accordance with a second aspect of the present invention there is provided
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.

In accordance with a third aspect of the present invention there is provided

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.

In accordance with a fourth aspect of the present invention there is provided

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

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