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(54) Title: RECOMBINANT RACCOONPOX VIRUS AND USES THEREOF AS A VACCINE IN MAMMALIAN AND AVIAN SPECIES (57) Abstract The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region, the HindIII "M" genomic region, or HindIII "N" genomic region of the raccoonpox virus genome. The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a deletion in a raccoonpox virus host range gene of the viral genome. The present invention provides a homology vector for producing a recombinant raccoonpox virus by inserting a foreign DNA sequence into the raccoonpox virus genome. The present invention provides a recombinant raccoonpox virus which is useful as a vaccine in mammalian and avian species.		

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RECOMBINANT RACCOONPOX VIRUS AND USES THEREOF AS A VACCINE
IN MAMMALIAN AND AVIAN SPECIES

5 This application claims priority of U.S. Serial No. 09/113, 750, filed July 10, 1998 the content of which is hereby incorporated by reference.

10 Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these references may be found at the end of the specification immediately preceding the Sequence Listing. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

Background of the Invention

20 Raccoonpox virus (RPV) belongs to the orthopoxvirus family, like vaccinia virus, but has an advantage in that it is naturally attenuated and appears to be native to North America. Unlike swinepox virus whose infection in non-porcine species is non-productive, raccoonpox virus infection is productive, but may not spread systemically like vaccinia virus.

25 Raccoonpox virus was originally isolated from the upper respiratory tract of 2 of 92 apparently healthy raccoons in 1962 in Maryland (1). Significant HI (hemagglutinating-inhibiting) antibody titers for RPV were demonstrated in 23% [22/92] of raccoon sera indicating that RPV is indigenous in North America (2). Serological surveys testing sera from 593
30 raccoons in Delaware demonstrated that 4% were positive for

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RPV neutralizing antibodies, 12% were positive by ELISA and 2.5% of the sera were positive by both tests (10). RPV is classified as a orthopoxvirus based upon biological, serological and biophysical methods. RPV and volepox are the only orthopoxviruses thought to be native to North America. The *Hind* III DNA restriction map of RPV is the most divergent from other orthopoxvirus DNA maps, which are highly conserved, indicating that RPV has diverged from the other group members (4,6). Of 29 *Hind*III fragments from the RPV genome, none comigrated with *Hind*III fragments that were common to other examined orthopoxvirus DNA. RPV is naturally attenuated (10-100X) and avirulent compared with wild type vaccinia virus. There are four published RPV DNA sequences which include a *Sal* I end fragment (2.2 kb) (5), a hemagglutinin gene (1.5 kb) (7), a protein phosphatase sequence (0.5 kb; H1L) (15), and the TK gene (834 bp) (16). RPV TK shows 87% amino acid homology to TK of vaccinia and 84.3% nucleotide homology. RPV HA shows 53% amino acid homology with vaccinia HA and 66% nucleotide homology. No other information regarding the genomic organization of RPV is known.

To date, recombinant raccoon poxviruses have been generated by insertion of foreign genes such as rabies virus glycoprotein into the TK locus of raccoon poxvirus (7-14). RPV recombinants expressing foreign viral antigens have been shown to elicit host protective immune responses in non-raccoon species including dogs, cats, and sheep with no negative effects observed.

The present invention provides a recombinant raccoon pox virus comprising a foreign DNA inserted into the *Hind*III M region, *Hind*III N region or *Hind*III U region of the raccoonpox virus genome. The recombinant raccoon poxvirus is useful as a vaccine in mammalian and avian species. Prior to the present invention, it was unknown whether non-essential regions

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existed in the HindIII M, HindIII N, or HindIII U regions, and whether a stable recombinant raccoonpox virus could be isolated with foreign DNA insertions into these regions.

5 Recombinant Raccoonpox Viruses derived from Herman Strain (ATCC VR-838) S-RPV-002, S-RPV-008 and SRPV-009 were deposited on February 10, 1998, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of
10 Microorganisms for the Purposes of Patent Procedure and were accorded ATCC Accession Nos. VR-2597, VR-2598 and VR-2599, respectively.

Summary of the Invention

15 The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region of the raccoonpox region of the raccoonpox virus genome.

20 The present invention provides a recombinant swinepox virus comprising a swinepox virus genome which contains a foreign DNA sequence inserted into a non-essential region of the raccoonpox virus genome, wherein the foreign DNA sequence is a host range gene selected from
25 the group consisting of raccoonpox virus K1L and raccoonpox virus C7L.

The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign virus viral genome which contains a

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foreign DNA sequence inserted into a non-essential region within the HindIII "N" genomic region of the raccoonpox virus genome.

5 The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "M" genomic region of the raccoonpox virus genome.

10 The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a deletion in a raccoonpox virus host range gene of the viral genome, wherein the raccoonpox virus host range gene is selected from the group consisting of C7L, C6L, C5L, C4L, C3L, C2L, C1L, N1L, N2L, M1L, M2L,
15 and K1L.

The present invention provides a homology vector for producing a recombinant raccoonpox virus by inserting a foreign DNA sequence into the raccoonpox virus genome which comprises a double-stranded DNA molecule
20 consisting of double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from an animal pathogen. Located at one end of the foreign DNA sequence, is double-stranded feline virus genomic DNA homologous to the genomic DNA located at one side of a
25 non-essential site of the raccoonpox viral genomic DNA. Located at the other end of the foreign DNA sequence, is double-stranded raccoonpox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.

Brief Description of the Figures

Figure 1: Restriction endonuclease map of raccoonpox virus HindIII "U" genomic fragment (approximately 2055 base pairs) (SEQ ID NO. 1). Two open reading frames (ORFs) are the O1L vaccinia homologue (SEQ ID NO. 3) and the E11L vaccinia homologue (SEQ ID NO. 2). (17) These two ORFs are non-essential for viral replication and suitable for insertion of foreign DNA. Restriction endonuclease sites in the RPV HindIII U genomic fragment are shown as suitable insertion sites. Examples of recombinant raccoonpox of the present invention have foreign DNA inserted into the XbaI site at nucleotide 1065 of the HindIII U fragment.

Figure 2: Restriction endonuclease map of raccoonpox virus HindIII "W", "T", and "P", genomic fragments (approximately 8300 base pairs) (SEQ ID NO. 5, 12). At least seven open reading frames (ORFs) within this region are the N1L vaccinia homologue (SEQ ID NO. 6), N2L vaccinia homologue (SEQ ID NO. 7), M1L vaccinia homologue (SEQ ID NO. 8), M2L vaccinia homologue (SEQ ID NO. 9), K1L vaccinia homologue (SEQ ID NO. 10), K2L vaccinia homologue (SEQ ID NO. 11), and the K7R vaccinia homologue (SEQ ID NO. 13). (17) These seven ORFs are non-essential for viral replication and suitable for insertion of foreign DNA. Restriction endonuclease sites in the RPV HindIII W, T and P genomic fragments are shown as suitable insertion

sites.

Figure 3: Restriction endonuclease map of raccoonpox virus HindIII "N" genomic fragment (approximately 5,100 base pairs). At least two open reading frames (ORFs) are the D1L cowpox virus homologue (18) and the B22R variola virus homologue. These two ORFs are non-essential for viral replication and suitable for insertion of foreign DNA. Restriction endonuclease sites in the RPV HindIII N genomic fragment are shown as suitable insertion sites. Examples of recombinant raccoonpox of the present invention have foreign DNA inserted into the EcoRV site at approximately nucleotide 1500 (S-RPV-009) or SnaBI site at approximately nucleotide 2200 (S-RPV-008) of the HindIII U fragment.

Figure 4: Restriction endonuclease map of raccoonpox virus HindIII "M" genomic fragment (approximately 6,000 base pairs). At least three open reading frames (ORFs) within this region are the D1L cowpox virus homologue (18) the C17L/B23R vaccinia virus homologue (17) and the D1L variola virus homologue (17). These three ORFs are non-essential for viral replication and suitable for insertion of foreign DNA. Restriction endonuclease sites in the RPV HindIII M genomic fragment are shown as suitable insertion sites. Examples of recombinant raccoonpox of the present invention have foreign DNA inserted into the HpaI site at approximately

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nucleotide 4100 (S-RPV-010) of the HindIII M fragment.

Figure 5: DNA sequence of the synthetic pox viral promoter LP2EP2 and position of unique EcoRI and BamHI sites (SEQ ID NO. 28) for insertion of foreign DNA under the control of the LP2EP2 promoter in homology vector 919-16.12. The colorimetric marker for positive selection of recombinant raccoonpox virus is the E. coli uidA gene. Recombinant raccoonpox virus, S-RPV-011, was constructed to contain this DNA sequence.

Figure 6: DNA sequence of the synthetic pox viral promoter LP2EP2 and position of unique EcoRI and BamHI sites (SEQ ID NO. 29) for insertion of foreign DNA under the control of the LP2EP2 promoter in homology vector 902-49.46, 902-67.1, 902-67.14, and 902-67.27. The colorimetric marker for positive selection of recombinant raccoonpox virus is the E. coli lacZ gene. Recombinant raccoonpox viruses, S-RPV-007, S-RPV-008, S-RPV-009, S-RPV-010, was constructed to contain this DNA sequence in different insertion regions of the recombinant raccoonpox virus.

Figure 7: DNA sequence of the raccoonpox virus EllL promoter and location of the translation start codon. Direction of transcription is from right to left. (SEQ ID NO. 30) The raccoonpox virus EllL promoter is useful for expression of foreign DNA in recombinant poxvirus vectors, including, but not limited

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to raccoonpox, swinepox, avipox, fowlpox, canarypox, cowpox, capripox, and vaccinia virus.

Figure 8: Restriction endonuclease map of raccoonpox virus HindIII "S" genomic fragment (approximately 2,375 base pairs). At least two open reading frames (ORFs) are the B19R vaccinia virus homologue (Surface antigen; IL-1 receptor) (17) and the B18R variola virus homologue (787 amino acids) (17); B20R vaccinia virus homologue (127 amino acids) (17). These three ORFs are non-essential for viral replication and suitable for insertion of foreign DNA. Restriction endonuclease sites in the RPV HindIII S genomic fragment are shown as suitable insertion sites. Examples of recombinant raccoonpox of the present invention have foreign DNA inserted into the HpaI site at approximately nucleotide 4100 (S-RPV-010) of the HindIII M fragment.

Figure 9: DNA sequence of the swinepox virus I5L promoter and location of the translation start codon. Direction of transcription is from right to left. (SEQ ID NO. 58) The swinepox virus I5L promoter is useful for expression of foreign DNA in recombinant poxvirus vectors, including, but not limited to raccoonpox, swinepox, avipox, fowlpox, canarypox, cowpox, capripox, and vaccinia virus.

Detailed Description of the Invention

The present invention provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region of the raccoonpox region of the raccoonpox virus genome. The present invention is useful for the expression of a foreign DNA or genes from other pathogens for protection against disease. Recombinant raccoonpox virus expressing a foreign DNA from avian or mammalian pathogens are useful as vaccines in avian or mammalian species including but not limited to feline, canine, equine, bovine, caprine, porcine, and primate, including human.

As defined herein, "viral genome" means the entire DNA which naturally occurs in the virus. As defined herein, "foreign DNA" or "foreign gene" means any DNA or gene that is exogenous to a genome.

In one embodiment of the present invention, the foreign DNA sequence is inserted into an O1L open reading frame. In another embodiment, the foreign DNA is inserted into an E11 open reading frame. In another embodiment, the foreign DNA sequence is inserted into an intergenic region. In another embodiment, the foreign DNA sequence is inserted into an XbaI restriction endonuclease site.

A further embodiment of the recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region of the raccoonpox virus genome, further comprises the

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foreign DNA sequence inserted into the HindIII "U" genomic region, wherein the foreign DNA is capable of being expressed in a recombinant raccoonpox virus host cell.

5 One embodiment of the present invention is wherein the foreign DNA sequence encodes a screenable marker. Another embodiment is wherein the screenable marker is *E. coli* beta-galactosidase or *E. coli* beta-glucuronidase. A further embodiment is wherein the
10 foreign DNA sequence encodes an antigenic polypeptide. Another embodiment of the present invention is wherein the antigenic polypeptide when introduced into the host cell, induces production of protective antibodies against a feline disease-causing agent from which the
15 antigenic polypeptide is derived or derivable. A further embodiment of the present invention is wherein the antigenic polypeptide is derived or derivable from a group consisting of feline pathogen, canine pathogen, equine pathogen, bovine pathogen, caprine pathogen,
20 avian pathogen, porcine pathogen, or human pathogen.

Another embodiment of the invention is wherein the foreign DNA sequence is selected from a group consisting of swine influenza virus hemagglutinin, swine influenza virus neuraminidase, porcine reproductive and
25 respiratory virus (PRRS) ORF2, PRRS ORF3, PRRS ORF4, PRRS ORF5, PRRS ORF6, PRRS ORF7, porcine parvovirus VP2.

Another embodiment of the invention is wherein the foreign gene is under control of an endogenous upstream, raccoonpox virus promoter. A further embodiment of the
30 present invention is wherein promoter is selected from a group consisting of the raccoonpox virus E11L promoter and the raccoonpox virus O1L promoter. Another

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embodiment is wherein the foreign gene is under control of a heterologous upstream promoter.

5 A further embodiment of the invention is wherein the promoter is selected from a group consisting of the synthetic pox late promoter 1 (LP1), the synthetic pox early promoter 1 (EP1), the synthetic early promoter 2 (EP2), the synthetic pox late promoter 2 early promoter 2 (LP2EP2), the synthetic pox late promoter 1 (LP1), the synthetic pox late promoter 2 (LP2), and the swinepox virus I5L promoter.

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One embodiment of the invention is a recombinant raccoonpox virus designated S-RPV-001, designated S-RPV-002, designated S-RPV-003, designated S-RPV-004, designated S-RPV-005, designated S-RPV-006, designated S-RPV-007, designated S-RPV-011, designated S-RPV-012, designated S-RPV-013, designated S-RPV-015, designated S-RPV-016, designated S-RPV-017, designated S-RPV-018, designated S-RPV-019, designated S-RPV-020, or designated S-RPV-028.

15

20 The present invention also provides for a recombinant swinepox virus of comprising a swinepox virus genome which contains a foreign DNA sequence inserted into a non-essential region of the swinepox virus genome, wherein the foreign DNA sequence is a host range gene selected from the group consisting of raccoonpox virus K1L and raccoonpox virus C7L. One embodiment of the present invention is the recombinant swinepox virus further comprising a second foreign DNA sequence inserted into a non-essential region within the swinepox virus genome. One embodiment of the present invention is the recombinant swinepox virus designated S-SPV-248. Another embodiment of the present invention is the

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recombinant swinepox virus designated S-SPV-249.

The present invention also provides a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted
5 into a non-essential region within the HindIII "N" genomic region of the raccoonpox virus genome. A further embodiment of the present invention is wherein the foreign DNA sequence is inserted into an SnaBI restriction endonuclease site. Another embodiment of
10 the present invention is wherein the foreign DNA sequence is inserted into an EcoRV restriction endonuclease site.

A preferred embodiment of the present invention is a recombinant raccoonpox virus designated S-RPV-008 or
15 designated S-RPV-009.

The present invention provides for a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "M"
20 genomic region of the raccoonpox virus genome. One embodiment of the present invention is wherein the foreign DNA sequence is inserted into an HpaI restriction endonuclease site. Another embodiment of the present invention is wherein the recombinant virus
25 is designated S-RPV-010.

The present invention also provides for a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a deletion in a raccoonpox virus host range gene of the viral genome, wherein the
30 raccoonpox virus host range gene is selected from the group consisting of C7L, C6L, C5L, C4L, C3L, C2L, C1L,

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N1L, N2L, M1L, M2L, and K1L. One embodiment of the present invention is a recombinant raccoonpox virus further comprising a second foreign DNA sequence inserted into a non-essential region within the raccoonpox virus genome.

The present invention also provides for a homology vector for producing a recombinant raccoonpox virus by inserting a foreign DNA sequence into the raccoonpox virus genome which comprises a double-stranded DNA molecule consisting of: (a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from an animal pathogen; (b) at one end of the foreign DNA sequence, double-stranded feline virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the raccoonpox viral genomic DNA; (c) at the other end of the foreign DNA sequence, double-stranded raccoonpox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.

In one embodiment of the homology vector the antigenic polypeptide is selected from a group consisting of swine influenza virus hemagglutinin, swine influenza virus neuraminidase, porcine reproductive and respiratory virus (PRRS) ORF2, PRRS ORF3, PRRS, ORF4, PRRS ORF5, PRRS ORF6, PRRS ORF7, porcine parvovirus VP2, *E. coli* beta-galactosidase gene., *E. Coli* beta-glucuronidase gene.

A further embodiment is a homology vector designated Homology Vector 902-16.2. Another embodiment is a homology vector designated Homology Vector 902-19.18. A further embodiment is a homology vector designated Homology Vector 902-49.5. A further embodiment is a

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homology vector designated Homology Vector 902-49.14.
A further embodiment is a homology vector designated
Homology Vector 902-49.23.

Another embodiment is a homology vector designated
5 Homology Vector 902-49.34. A further embodiment is a
homology vector designated Homology Vector 934-65.1, or
Homology Vector 934-64.2 or Homology Vector 938-94.1 or
Homology Vector 938-94.25 or Homology Vector 950-13.4 or
Homology Vector 936-87.2 or Homology Vector 950-16.34.

10 The present invention provides for a host cell infected
with the recombinant raccoonpox virus comprising a
raccoonpox virus viral genome which contains a foreign
DNA sequence inserted into a non-essential region within
the HindIII "U" genomic region of the raccoonpox virus
15 genome. One embodiment of the present invention is
wherein the host cell is mammalian cell.

The present invention also provides for a vaccine
against an animal pathogen which comprises an effective
immunizing amount of the recombinant raccoonpox virus
20 and a suitable carrier.

The present invention also provides for a method of
immunizing an animal against an animal pathogen which
comprises administering to the animal an effective
immunizing dose of the vaccine.

25 The present invention also provides for a method of
immunizing an animal against a feline pathogen which
comprises administering to the animal an effective
immunizing dose of the vaccine.

The present invention provides for a recombinant

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5 raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region of the raccoonpox region of the raccoonpox virus genome.

In one embodiment the foreign DNA sequence is inserted into an O1L open reading frame.

10 In another embodiment the foreign DNA sequence is inserted into an E11L open reading frame.

In one embodiment the foreign DNA sequence is inserted into an intergenic region.

In a further embodiment the foreign DNA sequence is inserted into an XbaI restriction endonuclease site.

15 In another embodiment the recombinant raccoonpox virus offurther comprises the foreign DNA sequence inserted into the HindIII "U" genomic region, wherein the foreign DNA is capable of being expressed in a recombinant raccoonpox virus host cell.

20 In another embodiment the foreign DNA sequence encodes a screenable marker.

In another embodiment the screenable marker is E. coli beta-galactosidase.

25 In another embodiment the screenable marker is E. coli beta-glucuronidase.

In one embodiment the foreign DNA sequence encodes an antigenic polypeptide.

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In another embodiment the antigenic polypeptide when introduced into the host cell, induces production of protective antibodies against a feline disease-causing agent from which the antigenic polypeptide is derived or derivable.

In another embodiment the antigenic polypeptide is derived or derivable from a group consisting of feline pathogen, canine pathogen, equine pathogen, bovine pathogen, caprine pathogen, avian pathogen, porcine pathogen, or human pathogen.

In another embodiment the foreign DNA sequence is selected from a group consisting of swine influenza virus hemagglutinin, swine influenza virus neuraminidase, porcine reproductive and respiratory virus (PRRS) ORF2, PRRS ORF3, PRRS ORF4, PRRS ORF5, PRRS ORF6, PRRS ORF7, porcine parvovirus VP2.

In another embodiment the foreign gene is under control of an endogenous upstream, raccoonpox virus promoter.

In another embodiment the promoter is selected from a group consisting of the raccoonpox virus E11L promoter and the raccoonpox virus O1L promoter.

In another embodiment the foreign gene is under control of a heterologous upstream promoter.

In another embodiment the promoter is selected from a group consisting of the synthetic pox late promoter 1 (LP1), the synthetic pox early promoter 1 (EP1), the synthetic pox early promoter 2 (EP2), the synthetic pox late promoter 2 early promoter 2 (LP2EP2), the synthetic pox late promoter 1 (LP1), the synthetic pox late

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promoter 2 (LP2), and the swinepox virus I5L promoter.

The present invention provides for a recombinant raccoonpox virus designated S-RPV-001, S-RPV-002, S-RPV-003 S-RPV-004, S-RPV-005, S-RPV-006, S-RPV-007, S-RPV-011, S-RPV-012, S-RPV-013, S-RPV-015, S-RPV-016, S-RPV-017, S-RPV-018, S-RPV-019, S-RPV-020, or S-RPV-028.

The present invention provides for a recombinant swinepox virus comprising a swinepox virus genome which contains a foreign DNA sequence inserted into a non-essential region of the raccoonpox virus genome, wherein the foreign DNA sequence is a host range gene selected from the group consisting of raccoonpox virus K1L and raccoonpox virus C7L.

In one embodiment the recombinant swinepox virus of further comprises a second foreign DNA sequence inserted into a non-essential region within the swinepox virus genome.

In one embodiment the recombinant swinepox virus is designated S-SPV-248 or S-SPV-249.

The present invention provides for a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "N" genomic region of the raccoonpox virus genome.

In one embodiment the foreign DNA sequence is inserted into an EcoRI restriction endonuclease site.

In another embodiment the foreign DNA sequence is

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inserted into an SnaBI restriction endonuclease site.

The present invention provides for a recombinant raccoonpox virus of designated S-RPV-008 or S-RPV-009.

5 The present invention provides for a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "M" genomic region of the raccoonpox virus genome.

10 In one embodiment the foreign DNA sequence is inserted into an HpaI restriction endonuclease site.

The present invention provides for a recombinant raccoonpox virus of designated S-RPV-010.

15 The present invention provides for a recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a deletion in a raccoonpox virus host range gene of the viral genome, wherein the raccoonpox virus host range gene is selected from the group consisting of C7L, C6L, C5L, C4L, C3L, C2L, C1L, N1L, N2L, M1L, M2L, and K1L.

20 In one embodiment the recombinant raccoonpox virus of, further comprises a second foreign DNA sequence inserted into a non-essential region within the raccoonpox virus genome.

25 The present invention provides for a homology vector for producing a recombinant raccoonpox virus by inserting a foreign DNA sequence into the raccoonpox virus genome which comprises a double-stranded DNA molecule consisting of double-stranded foreign DNA sequence

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encoding an antigenic polypeptide derived from an animal pathogen; at one end of the foreign DNA sequence, double-stranded feline virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the raccoonpox viral genomic DNA; at the other end of the foreign DNA sequence, double-stranded raccoonpox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.

In one embodiment the antigenic polypeptide is selected from a group consisting of swine influenza virus hemagglutinin, swine influenza virus neuraminidase, porcine reproductive and respiratory virus (PRRS) ORF2, PRRS ORF3, PRRS, ORF4, PRRS ORF5, PRRS ORF6, PRRS ORF7, porcine parvovirus VP2, E. coli beta-galactosidase gene., and E. Coli beta-glucuronidase gene.

The present invention provides for a homology vector designated Homology Vector 902-16.2, Homology Vector 902-19.18, Homology Vector 902-49.5, Homology Vector 902-49.14, Homology Vector 902-49.23, Homology Vector 902-49.34, Homology Vector 934.65-1, Homology Vector 934-64.2, Homology Vector 938-94.1 Homology Vector 938-94.25, Homology Vector 950-13.4, Homology Vector 936-87.2, Homology Vector 950-16.34,

The present invention provides for a host cell infected with a recombinant 'raccoonpox comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region of the raccoonpox region of the raccoonpox virus genome.

In one embodiment the host cell is mammalian cell.

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The present invention provides for a vaccine against an animal pathogen which comprises an effective immunizing amount of a recombinant raccoonpox virus and a suitable carrier.

- 5 The present invention provides for a method of immunizing an animal against an animal pathogen which comprises administering to the animal an effective immunizing dose of a recombinant raccoonpox virus and a suitable carrier.
- 10 The present invention provides for a method of immunizing an animal against a feline pathogen which comprises administering to the animal an effective immunizing dose of a recombinant raccoonpox virus and a suitable carrier.
- 15 The invention provides a recombinant raccoonpox virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is useful to prevent disease in porcine, bovine, feline, canine, avian, equine, caprine and human species.
- 20 The invention provides a recombinant raccoonpox virus capable of replication which contains foreign DNA encoding an antigenic polypeptide which is or is from pseudorabies virus (PRV) g50 (gD), pseudorabies virus (PRV) gII (gB), Pseudorabies virus (PRV) gIII (gC),
25 pseudorabies virus (PRV) glycoprotein H, pseudorabies virus (PRV) glycoprotein E, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein,
30 Serpulina hydodysenteriae protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, swine influenza

hemagglutinin or , swine influenza neuraminidase.
Preferably, the antigenic polypeptide is Pseudorabies
Virus (PRV) g50 (gD). Preferably the antigenic
polypeptide is swine influenza hemagglutinin or swine
5 influenza neuraminidase.

The present invention further provides an antigenic
polypeptide which includes, but is not limited to: hog
cholera virus gE1, hog cholera virus gE2, swine
influenza virus hemagglutinin, neurominidase, matrix and
10 nucleoprotein, pseudorabies virus gB, gC and gD, and
porcine reproductive and respiratory disease (PRRS)
virus ORF2, ORF3, ORF4, ORF5, ORF6 and ORF7. Preferably
the antigenic polypeptide is PRRS, ORF2, ORF3, ORF4,
ORF5, ORF6, and ORF 7.

15 The invention further provides a recombinant raccoonpox
virus capable of replication which contains foreign DNA
encoding an antigenic polypeptide which is or is from
Serpulina hyodysenteriae, Foot and Mouth Disease Virus,
Hog Cholera Virus, Swine Influenza Virus, African Swine
20 Fever Virus or Mycoplasma hyopneumoniae.

The invention further provides a recombinant raccoonpox
virus capable of replication which contains foreign DNA
encoding an antigenic polypeptide which is or is from
feline leukemia virus, feline immunodeficiency virus and
25 *Dirofilaria immitis* (heartworm). Additional antigens
from disease causing microorganisms are expressed in
raccoonpox virus. Disease causing microorganisms in cats
include, but are not limited to *Dirofilaria immitis* p39
and 22kD antigens, feline infectious peritonitis virus,
30 calicivirus, rabies virus, feline parvovirus
(panleukopenia virus), feline coronavirus and feline
Chlamydia, *Toxoplasma gondii*. Disease causing

microorganisms in dogs include, but are not limited to canine distemper, canine adenovirus type 1 (hepatitis), adenovirus type 2 (respiratory disease), parainfluenza, leptospira canicola, icterohemorrhagia, parvovirus, coronavirus, borrelia burgdorferi, canine herpesvirus, bordetella bronchiseptica and rabies virus.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non-essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious laryngotracheitis virus and is capable of being expressed in a host infected by the recombinant raccoonpox virus. Examples of such antigenic polypeptide are infectious laryngotracheitis virus glycoprotein G, glycoprotein D and glycoprotein I, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, NDV fusion protein (F).

In one embodiment of the recombinant raccoonpox virus the foreign DNA sequence encodes a cytokine. In another embodiment the cytokine is chicken myelomonocytic growth factor (cMGF) or chicken interferon (cIFN). Cytokines include, but are not limited to: transforming growth factor beta, epidermal growth factor family, fibroblast growth factors, hepatocyte growth factor, insulin-like growth factor, vascular endothelial growth factor, interleukin 1, IL-1 receptor antagonist, interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, IL-6 soluble receptor, interleukin-7, interleukin-8, interleukin-9, interleukin-10, interleukin-11, interleukin-12, interleukin-13, interleukin-14, interleukin-15, interleukin-16, angiogenin, chemokines, colony stimulating factors,

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granulocyte- macrophage colony stimulating factors, erythropoietin, interferon, interferon gamma, c-kit ligand, leukemia inhibitory factor, oncostatin M, pleiotrophin, secretory leukocyte protease inhibitor, stem cell factor, tumor necrosis factors, and soluble TNF receptors. These cytokines are from humans, bovine, equine, feline, canine, porcine or avian.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non- essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from a human pathogen and is capable of being expressed in a host infected by the recombinant raccoonpox virus.

Recombinant RPV expressing cytokines is used to enhance the immune response either alone or when combined with vaccines containing cytokines or antigen genes of disease causing microorganisms.

Antigenic polypeptide of a human pathogen which are derived from human herpesvirus include, but are not limited to: hepatitis B virus and hepatitis C virus hepatitis B virus surface and core antigens, hepatitis C virus, human immunodeficiency virus, herpes simplex virus-1, herpes simplex virus-2, human cytomegalovirus, Epstein-Barr virus, Varicella-Zoster virus, human herpesvirus-6, human herpesvirus-7, human influenza, measles virus, hantaan virus, pneumonia virus, rhinovirus, poliovirus, human respiratory syncytial virus, retrovirus, human T-cell leukemia virus, rabies virus, mumps virus, malaria (*Plasmodium falciparum*), *Bordetella pertussis*, *Diphtheria*, *Rickettsia prowazekii*, *Borrelia burgdorferi*, Tetanus toxoid, malignant tumor

antigens.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non- essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes a cytokine capable of stimulating an immune in a host infected by the recombinant raccoonpox virus and is capable of being expressed in the host infected.

The antigenic polypeptide of an equine pathogen can derived from equine influenza virus, or equine herpesvirus. In one embodiment the antigenic polypeptide is equine influenza neuraminidase or hemagglutinin. Examples of such antigenic polypeptide are equine influenza virus type A/Alaska 91 neuraminidase, equine influenza virus type A/Prague 56 neuraminidase, equine influenza virus type A/Miami 63 neuraminidase, equine influenza virus type A/Kentucky 81 neuraminidase, equine influenza virus type A/Kentucky 92 neuraminidase equine herpesvirus type 1 glycoprotein B, equine herpesvirus type 1 glycoprotein D, Streptococcus equi, equine infectious anemia virus, equine encephalitis virus, equine rhinovirus and equine rotavirus.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non- essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from bovine respiratory syncytial virus or bovine parainfluenza virus, and is capable of being expressed in a host infected by the recombinant raccoonpox virus.

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For example, the antigenic polypeptide of derived from infectious bovine rhinotracheitis virus gE, bovine respiratory syncytial virus equine pathogen can derived from equine influenza virus is bovine respiratory syncytial virus attachment protein (BRSV G), bovine respiratory syncytial virus fusion protein (BRSV F), bovine respiratory syncytial virus nucleocapsid protein (BRSV N), bovine parainfluenza virus type 3 fusion protein, and the bovine parainfluenza virus type 3 hemagglutinin neuraminidase.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non- essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes an antigenic polypeptide derived from infectious bovine rhinotracheitis virus and is capable of being expressed in a host infected by the recombinant raccoonpox virus. Examples of such antigenic polypeptide are infectious bovine rhinotracheitis virus glycoprotein E, glycoprotein G, glycoprotein gD and glycoprotein gI.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non- essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes bovine viral diarrhea virus (BVDV) glycoprotein 48 or glycoprotein 53, and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant raccoonpox virus.

The present invention further provides a recombinant raccoonpox virus which comprises a foreign DNA sequence inserted into a non- essential site of the raccoonpox genome, wherein the foreign DNA sequence encodes an

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antigenic polypeptide derived from infectious bursal disease virus and wherein the foreign DNA sequence is capable of being expressed in a host infected by the recombinant raccoonpox virus. Examples of such antigenic polypeptide are infectious bursal disease virus polyprotein and VP2.

The present invention further provides a recombinant raccoonpox virus in which the foreign DNA sequence encodes an antigenic polypeptide which includes, but is not limited to: MDV gA, MDV gB, MDV gD, NDV HN, NDV F, ILT gB, ILT gI, ILT gD, IBDV VP2, IBDV VP3, IBDV VP4, IBDV polyprotein, IBV spike, IBV matrix, avian encephalomyelitis virus, avian reovirus, avian paramyxovirus, avian influenza virus, avian adenovirus, fowl pox virus, avian coronavirus, avian rotavirus, chick anemia virus, Salmonella spp. E. coli, Pasteurella spp., Bordetella spp., Eimeria spp., Histomonas spp., Trichomonas spp., Poultry nematodes, cestodes, trematodes, poultry mites/lice, and poultry protozoa.

This invention is illustrated in the Experimental Detail sections which follow. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

Experimental Details

Preparation of raccoonpox virus stock samples

Raccoonpox virus (RPV) isolate ATCC VR-838 was used for preparation of raccoonpox virus stock samples and raccoonpox virus genomic DNA. Another RPV isolate available is V71-I-85A from Center for Disease Control (CDC; Atlanta, GA). Raccoonpox virus (RPV) samples were prepared by infecting VERO cells, CRFK cells or MDCK cells at a multiplicity of infection of 0.01 PFU/cell in Dulbecco's Modified Eagle's Medium containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components were obtained from Sigma or equivalent supplier, and hereafter are referred to as DMEM negative medium). Prior to infection, the cell monolayers were washed once with DMEM negative medium to remove traces of fetal bovine serum. The RPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T225 cm flask) was then allowed to absorb onto the cell monolayer for two hours, being redistributed every half hour. After this period, the original inoculum was brought up to the recommended volume with the addition of complete DMEM medium (DMEM negative medium plus 5% fetal bovine serum). The plates were incubated at 37°C in 5% CO₂ until cytopathic effect was complete. The medium and cells were harvested and frozen in a 50 ml conical screw cap tube at -70°C. Upon thawing at 37°C, the virus stock was aliquoted into 1.0 ml vials and refrozen at -70°C. The titers were usually about 10⁵-10⁶ PFU/ml.

Preparation of RPV DNA

For raccoonpox virus DNA isolation, a confluent

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monolayer of VERO cells in a T225 cm² flask was infected at a multiplicity of 0.1 with raccoonpox virus (ATCC VR-838) and incubated 3-5 days until the cells were showing 100% cytopathic effect. The infected cells were then harvested by scraping the cells into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in 1.0 ml Phosphate Buffer Saline (PBS: 1.5g Na₂HPO₄, 0.2g KH₂PO₄, 0.8g NaCl and 0.2g KCl per liter H₂O) (per T175) and subjected to two successive freeze-thaws (-70°C to 37° C). Upon the last thaw, the cells (on ice) were sonicated two times for 30 seconds each with 45 seconds cooling time in between. Cellular debris was then removed by centrifuging (Sorvall RC-5B superspeed centrifuge) at 3000 rpm for 5 minutes in a HB4 rotor at 4° C. RPV virions, present in the supernatant, were then pelleted by centrifugation at 15,000 rpm for 20 minutes at 4° C in a SS34 rotor (Sorvall) and resuspended in 10 mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60 minutes in a SW41 rotor (Beckman) at 4° C. The virion pellet was resuspended in 1.0 ml of 10 mM tris pH 7.5 and sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged 16,000 rpm for 60 minutes in a SW41 rotor at 4°C. The RPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4° C. The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces of sucrose and finally resuspended in 10 mM Tris pH 7.5. RPV DNA was then extracted from the purified virions by lysis (4 hours at 60°C) induced by the addition of EDTA,

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SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mg/ml, respectively. After digestion, three phenol:chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and incubation at -20°C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for 5 minutes at full speed. The supernatant was decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1 mM EDTA at 4°C.

10 Preparation of infected cell lysates

For cell lysate preparation, serum free medium was used. A confluent monolayer of cells (VERO, CRFK, or MDCK) in a 25 cm² flask or a 60 mm petri dish was infected with 100 µl of virus sample. After cytopathic effect was complete, the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. The cell pellet was resuspended in 250 µl of disruption buffer (2% sodium dodecyl sulfate, 2% β-mercapto-ethanol). The samples were sonicated for 30 seconds on ice and stored at -20°C.

Western blotting procedure

Samples of lysates and protein standards were run on a polyacrylamide gel according to the procedure of Laemmli (19). After gel electrophoresis the proteins were transferred and processed according to Sambrook et al. (1989) (20). The primary antibody was diluted 1:100 with 5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H₂O). The secondary antibody was alkaline phosphatase conjugated and diluted 1:1000 with TSA.

Molecular biological techniques

Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Sambrook et al. (1989) and *Current Protocols in Molecular Biology* (1992). (20,21) Except as noted, these were used with minor variation.

DNA sequencing

DNA sequencing was performed by fluorescent labeled dideoxy sequencing reactions using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AMPLITAQTM DNA polymerase; FS (Perkin-Elmer; per manufacturer's instructions) and electrophoresed on an Perkin-Elmer/Applied Biosystems automated DNA sequencer Model 373A according to manufacturer's instructions. Reactions using both the dGTP mixes and the dTTP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using DNASTar software.

Cloning with the polymerase chain reaction

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The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis, et al. (1990) (22). In general, amplified fragments were less than 500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. The primers used in each example are detailed in the descriptions of the construction of homology vectors below.

10 Homologous recombination procedure for generating recombinant RPV or SPV

This method relies upon the homologous recombination between the raccoonpox virus DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both raccoonpox virus DNA or swinepox virus DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of cells (CRFK, MDCK, ESK-4, PK15 or VERO) are infected with S-RPV-000 (ATCC VR-838) or S-SPV-001 at a multiplicity of infection of 0.01 PFU/cell to introduce replicating RPV or SPV (i.e. DNA synthesis) into the cells. The plasmid homology vector DNA is then transfected into these cells according to the INFECTION - TRANSFECTION PROCEDURE. The construction of homology vectors used in this example of the procedure is described below.

Infection - transfection procedure for RPV or SPV

6 cm plates of cells (CRFK, MDCK, ESK4, or VERO) about 80% confluent were infected with S-RPV-000 or SPV-001 at a multiplicity of infection of 0.01 PFU/cell in DMEM negative medium or ESK negative medium and incubated at

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37°C in a humidified 5% CO₂ environment for 2-3 hours. The transfection procedure used is essentially that recommended for LIPOFECTINTM Reagent (BRL). Briefly, for each 6 cm plate, 15 µg of plasmid DNA was diluted up to 100 µl with H₂O. Separately, 50 micrograms of LIPOFECTINTM Reagent was diluted to 100 µl with H₂O. The 100 µl of diluted LIPOFECTINTM Reagent was then added dropwise to the diluted plasmid DNA contained in a polystyrene 5 ml snap cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the virus inoculum was removed from the 6 cm plates and the cell monolayers washed once with either DMEM or ESK negative medium. Five ml of DMEM negative medium or ESK negative medium was then added to the plasmid DNA/LIPOFECTINTM mixture and the contents pipetted onto the cell monolayer. The cells were incubated overnight (about 16 hours) at 37°C in a humidified 5% CO₂ environment. The next day the 5 ml of DMEM negative medium was removed and replaced with 5 ml DMEM complete medium. The cells were incubated at 37°C in 5% CO₂ for 3-5 days until cytopathic effect from the virus was 80-100%. Virus was harvested as described above for the preparation of virus stocks. This stock was referred to as a transfection stock and was subsequently screened for recombinant virus by the BLUOGALTM SCREEN FOR RECOMBINANT RACCOONPOX VIRUS OR CPRG SCREEN FOR RECOMBINANT RACCOONPOX VIRUS.

Screen for recombinant RPV or SPV expressing β-galactosidase BLUOGALTM and CPRG assays) or β-glucuronidase (X-GLUCTM assay)

When the *E. coli* β-galactosidase (lacZ) marker gene was incorporated into a recombinant virus the plaques

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containing the recombinants were visualized by one of two simple methods. In the first method, the chemical BLUOGALTM (Life Sciences Technology, Bethesda, MD) was incorporated (200 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-galactosidase turned blue. The blue plaques were then picked onto fresh cells (MDCK, ESK4, CRFK or VERO) and purified by further blue plaque isolation. In the second method, CPRG (Boehringer Mannheim) was incorporated (400 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-galactosidase turned red. The red plaques were then picked onto fresh cells (MDCK, ESK4 CRFK or VERO) and purified by further red plaque isolation. In both cases viruses were typically purified with three to four rounds of plaque purification.

When the *E. coli* β- glucuronidase (*uidA*) marker gene was incorporated into a recombinant virus the plaques containing the recombinants were visualized by using the chromogenic substrate, X-beta-D-gluUA CHX (X-GLUCTM; 5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt; Biosynth AG; Switzerland) was incorporated (200 µg/ml) into the agarose overlay during the plaque assay, and plaques expressing active β-glucuronidase turned blue. The blue plaques were then picked onto fresh cells (MDCK, ESK4 CRFK or VERO) and purified by further blue plaque isolation.

Screen for foreign gene expression in recombinant RPV using black plaque assays

To analyze expression of foreign antigens expressed by recombinant raccoonpox viruses, monolayers of cells

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(MDCK, CRFK or VERO) were infected with recombinant RPV, overlaid with nutrient agarose media and incubated for 3-5 days at 37 C for plaque development to occur. The agarose overlay was then removed from the dish, the cells fixed with 100% methanol for 10 minutes at room temperature and the cells air dried. Fixation of the cells results in cytoplasmic antigen as well as surface antigen detection whereas specific surface antigen expression can be detected using non-fixed cells. The primary antibody was then diluted to the appropriate dilution with 1X blotto (5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H₂O) and incubated on the cell monolayer for 2 hours at room temperature. Unbound antibody was then removed by washing the cells three times with TS buffer at room temperature. The secondary antibody, a alkaline-phosphatase conjugate, was diluted 1:1000 with 1X blotto and incubated with the cells for 2 hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with TS buffer (6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl per liter H₂O) at room temperature. The cells were then incubated 15-30 minutes at room temperature with freshly prepared substrate solution (100 mM Tris HCl pH. 9.5, 100 mM NaCl, 5 mM MgCl₂, 0.3 mg/ml Nitro Blue Tetrazolium and 0.15 mg/ml 5-Bromo-4-chloro-3-Indoyl Phosphatas). Plaques expressing the correct antigen stain black. A fixer solution (20 mM Tris-HCl pH 2.9 and 1mM EDTA) was used to stop the color development reaction.

Procedure for purification of viral glycoproteins for use as diagnostics

Viral glycoproteins are purified using antibody affinity

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columns. To produce monoclonal antibodies, 8 to 10 week old BALB/c female mice are vaccinated intraperitoneally seven times at two to four week intervals with 10^7 PFU of raccoonpox virus recombinants. Three weeks after the
5 last vaccination, mice are injected intraperitoneally with 40 mg of the corresponding viral glycoprotein. Spleens are removed from the mice three days after the last antigen dose.

Splenocytes are fused with mouse NS1/Ag4 plasmacytoma
10 cells by the procedure modified from Oi and Herzenberg, (23). Splenocytes and plasmacytoma cells are pelleted together by centrifugation at $300 \times g$ for 10 minutes. One ml of a 50% solution of polyethylene glycol (m.w. 1300-1600) is added to the cell pellet with stirring
15 over one minute. Dulbecco's modified Eagles's medium (5ml) is added to the cells over three minutes. Cells are pelleted by centrifugation at $300 \times g$ for 10 minutes and resuspended in medium with 10% fetal bovine serum and containing 100 mM hypoxanthine, 0.4 mM aminopterin
20 and 16 mM thymidine (HAT). Cells (100 ml) are added to the wells of eight to ten 96-well tissue culture plates containing 100 ml of normal spleen feeder layer cells and incubated at 37°C . Cells are fed with fresh HAT medium every three to four days.

25 Hybridoma culture supernatants are tested by the ELISA ASSAY in 96-well microtiter plates coated with 100 ng of viral glycoprotein. Supernatants from reactive hybridomas are further analyzed by black-plaque assay and by Western Blot. Selected hybridomas are cloned
30 twice by limiting dilution. Ascetic fluid is produced by intraperitoneal injection of 5×10^6 hybridoma cells into pristane-treated BALB/c mice.

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Cell lysates from raccoonpox virus recombinants are obtained as described in PREPARATION OF INFECTED CELL LYSATES. The glycoprotein-containing cell lysates (100 mls) are passed through a 2-ml agarose affinity resin to which 20 mg of glycoprotein monoclonal antibody has been immobilized according to manufacturer's instructions (AFC Medium, New Brunswick Scientific, Edison, N.J.). The column is washed with 100 ml of 0.1% Nonidet P-40 in phosphate-buffered saline (PBS) to remove nonspecifically bound material. Bound glycoprotein is eluted with 100 mM carbonate buffer, pH 10.6 (24). Pre- and posteluted fractions are monitored for purity by reactivity to the RPV monoclonal antibodies in an ELISA system.

15 Elisa assay

A standard enzyme-linked immunosorbent assay (ELISA) protocol is used to determine the immune status of cattle following vaccination and challenge.

A glycoprotein antigen solution (100 ml at ng/ml in PBS) is allowed to absorb to the wells of microtiter dishes for 18 hours at 4°C. The coated wells are rinsed one time with PBS. Wells are blocked by adding 250 ml of PBS containing 1% BSA (Sigma) and incubating 1 hour at 37°C. The blocked wells are rinsed one time with PBS containing 0.02% Tween 20. 50 ml of test serum (previously diluted 1:2 in PBS containing 1% BSA) are added to the wells and incubated 1 hour at 37°C. The antiserum is removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 50 ml of a solution containing anti-bovine IgG coupled to horseradish peroxidase (diluted 1:500 in PBS containing 1% BSA, Kirkegaard and Perry Laboratories, Inc.) is added to

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visualize the wells containing antibody against the specific antigen. The solution is incubated 1 hour at 37 C, then removed and the wells are washed 3 times with PBS containing 0.02% Tween 20. 100 ml of substrate solution (ATBS, Kirkegaard and Perry Laboratories, Inc.) are added to each well and color is allowed to develop for 15 minutes. The reaction is terminated by addition of 0.1M oxalic acid. The color is read at absorbance 410nm on an automatic plate reader.

10 Strategy for the construction of synthetic pox viral promoters

For recombinant swinepox vectors synthetic pox promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. Three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the vaccinia virus (1, 7 and 8) were designed. Each cassette was designed to contain the DNA sequences defined in vaccinia flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each cassette such that inframe fusions could be made at either EcoRI or BamHI sites. A set of translational stop codons in all three reading frames and an early transcriptional termination signal were also engineered downstream of the inframe fusion site. DNA encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors (see Figures 5, 6, and 7).

30 Procedure for production of murine polyclonal antibodies to proteins expressed in recombinant raccoonpox virus

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Three groups of five mice each were vaccinated subcutaneously in the rear leg with 200 ul of a 1:1 mixture of infected cell lysate and Freund's complete adjuvant (S-RPV-003 (SIV NA), S-RPV-004 (PRRS ORF3) and S-RPV-005 (PRRS ORF4)). The infected cell lysates were prepared by harvesting a T225 flask Vero cells at 90%+ CPE. The infected cell pellets were washed once in PBS and finally resuspended in 1 ml of PBS. The 1 ml lysate was frozen at -70 C and thawed on ice. The 1 ml lysate was sonicated one time (lowest setting). The mice received two additional doses of cell lysates mixed with Freund's incomplete adjuvant at 3 weeks post vaccination and 6 weeks post vaccination. The mice were sacrificed at 8 weeks post vaccination. About 2 ml of serum was obtained from 5 mice. The serum was tested for SIV or PRRS specific antibodies by BLACK PLAQUE ASSAY.

Plasmid 880-96.43

The plasmid 880-96.43 was constructed for the purpose of inserting foreign DNA into raccoonpox virus. It comprises the approximately 2055 base pair HindIII U genomic fragment of raccoonpox virus (SEQ ID NO. 1). Two open reading frames within the HindIII U fragment are the vaccinia virus homologs (17) of the O1L ORF (SEQ ID NO. 2) and the E11L ORF (SEQ ID NO. 3). The O1L ORF and E11L ORF are non-essential and foreign DNA is inserted within these ORFs or in the intergenic region between the ORFs. Any restriction site within this region is useful as an insertion site for foreign DNA. A restriction enzyme site within this region which is not unique is altered by insertion of a DNA linker which converts the site to a unique restriction enzyme recognition sequence. Preferably the restriction enzyme site used for insertion of foreign DNA is an XbaI site

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at approximately nucleotide 1065 within the 2055 base pair HindIII U genomic fragment (SEQ ID NO. 1). The insertion site is within the O1L ORF between amino acids 459 and 460 of the open reading frame. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 2055 base pair HindIII U genomic fragment of raccoonpox virus (6) (SEQ ID NO. 1). Plasmid 880-96.43 was used to make homology vectors for insertion of foreign DNA in recombinant raccoonpox virus.

Plasmid 902-15.2

The plasmid 902-15.2 was constructed for the purpose of inserting foreign DNA into raccoonpox virus. It comprises the approximately 1801 base pair subfragment (SEQ ID NO. 4) of the HindIII U genomic fragment of raccoonpox virus. Two open reading frames within the HindIII U fragment are the vaccinia virus homologs (17) of the O1L ORF (SEQ ID NO. 2) and the E11L ORF (SEQ ID NO. 3). The O1L ORF and E11L ORF are non-essential and foreign DNA is inserted within these ORFs or in the intergenic region between the ORFs. Any restriction site within this region is useful as an insertion site for foreign DNA. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 1801 base pair HindIII subfragment of the HindIII U genomic fragment (6) synthesized by POLYMERASE CHAIN REACTION using plasmid 880-96.43 as a template. The primer upstream of the O1L and E1L ORFs was 5'-CCGAAGCTTCCGTGCTCCATCTATATAATATATTAAAC-3' (SEQ ID NO 14; 2/97.6). The primer downstream of the O1L and E11L ORFs

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was 5'-GAATAAGCTTCCCGTTACTTTAGTAAAATCTTTTACAAA-3' (SEQ ID NO 15; 2/97.7). A unique NotI synthetic linker was inserted within an XbaI site (Nucleotides 1098 to 1113; a unique NotI site is at nucleotide 1105; SEQ ID NO. 4)

5 The restriction enzyme site used for insertion of foreign DNA is an XbaI site at approximately nucleotide 1065 within the 2055 base pair HindIII U genomic fragment (SEQ ID NO. 1). The insertion site is within the O1L ORF between amino acids 459 and 460 of the open
10 reading frame. Plasmid 902-15.2 was used to make homology vectors for insertion of foreign DNA in recombinant raccoonpox virus.

Homology vector 902-16.2

The plasmid 902-16.2 was constructed for the purpose of
15 inserting foreign DNA into recombinant raccoonpox virus (RPV). It incorporates an E. coli β -galactosidase (lacZ) marker gene and a swine influenza virus (SIV) HA (H1N1) gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA.
20 Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. Direction of transcription of the SIV HA gene is opposite the direction of transcription of the lacZ gene and opposite to the direction of transcription of the RPV O1L ORF.
25 When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the
30 SIV HA (H1N1) gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21),

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by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM).
5 Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1721 base pair BamHI to BamHI restriction fragment synthesized by reverse transcription and
10 polymerase chain reaction (PCR) using genomic RNA from the SIV H1N1 strain (NVSL) To synthesize SIV HA (H1N1), the primer 5'-CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACATT-3'; 6/95.5) (SEQ ID NO 16) synthesized from the 5' end of the SIV HA (H1N1) gene, introduced an BamHI site at
15 the 5' end of the gene. The primer (5'-CTCTGGGATCCTAATTTTAAATACATATTCTGCACTGTA-3'; 6/95.6) (SEQ ID NO 17) was used for reverse transcription and PCR and synthesized from the 3' end of the SIV HA (H1N1) gene and introduced a BamHI site at the 3' end of the gene.
20 The PCR product was digested with BamHI to yield a fragment approximately 1721 base pairs in length corresponding to the SIV HA (H1N1) gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4
25 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U (6). The XbaI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

Homology vector 902-19.18

30 The plasmid 902-19.18 was constructed for the purpose of inserting foreign DNA into recombinant raccoonpox virus (RPV). It incorporates an E. coli β -galactosidase (lacZ) marker gene and a swine influenza virus (SIV)

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hemagglutinin (HA) (H1N1) gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. In contrast to homology vector 902-16.2 direction of transcription of the SIV HA gene is the same as the direction of transcription of the lacZ gene and the same as the direction of transcription of the RPV O1L ORF. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the SIV HA (H1N1) gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1721 base pair BamHI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from the SIV H1N1 strain (NVSL) To synthesize SIV HA (H1N1), the primer 5'-CCGAGGATCCGGCAATACTATTAGTCTTGCTATGTACAT-3'; 6/95.5) (SEQ ID NO 18) synthesized from the 5' end of the SIV HA (H1N1) gene, introduced an BamHI site at the 5' end of the gene. The primer (5'-CTCTGGATCCTAATTTAAATACATATTCTGCACTGTA-3'; 6/95.6) (SEQ ID NO 19) was used for reverse transcription and PCR and synthesized from the 3' end of the SIV HA (H1N1) gene and introduced a BamHI site at the 3' end of the gene.

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The PCR product was digested with BamHI to yield a fragment approximately 1721 base pairs in length corresponding to the SIV HA (H1N1) gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (11). Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U (6). The XbaI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

10 Homology vector 902-49.5

The plasmid 902-49.5 was constructed for the purpose of inserting foreign DNA into recombinant raccoonpox virus (RPV). It incorporates an E. coli β -galactosidase (lacZ) marker gene and a swine influenza virus (SIV) neuraminidase (NA) (H1N1) gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the SIV NA (H1N1) gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U

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(6). Fragment 2 is an approximately 1414 base pair EcoRI to BglIII restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from the SIV H1N1 strain (NVSL). To synthesize SIV NA (H1N1), the primer 5'-AATGAATTCAAATCAAAAAATAATAACCATGGGTCAAT-3'; 6/95.11) (SEQ ID NO 20) synthesized from the 5' end of the SIV NA (H1N1) gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-GGAAGATCTACTTGTCAATGGTGAATGGCAGATCAG-3'; 6/95.12) (SEQ ID NO 21) was used for reverse transcription and PCR and synthesized from the 3' end of the SIV NA (H1N1) gene and introduced a BglIII site at the 3' end of the gene. The PCR product was digested with EcoRI and BglIII to yield a fragment approximately 1414 base pairs in length corresponding to the SIV NA (H1N1) gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

Homology vector 902-49.14

The plasmid 902-49.14 was constructed for the purpose of inserting foreign DNA into recombinant raccoonpox virus (RPV). It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF3 gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING

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RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF3 gene is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF3, the primer 5'-TTCTGAATTCGGCTAATAGCTGTACATTCCTCCATATTT-3'; 1/96.7) (SEQ ID NO 22) synthesized from the 5' end of the PRRS ORF3 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-GGGGATCCTATCGCCGTACGGCACTGAGGG-3'; 1/96.8) (SEQ ID NO 23) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF3 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 768 base pairs in length corresponding to the PRRS ORF3 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

Homology vector 902-49.23

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The plasmid 902-49.23 was constructed for the purpose of inserting foreign DNA into recombinant raccoonpox virus (RPV). It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF4 gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the

10 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF4 gene is under the

15 control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an

20 approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an EcoRI to BamHI restriction

25 fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF4, the primer (5'-CCGAATTCGGCTGCGTCCCTTCTTTTCCTCATGG-3'; 1/96.11) (SEQ ID

30 NO 24) synthesized from the 5' end of the PRRS ORF4 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5' CTGGATCCTTCAAATTGCCAACAGAAATGGCAAAAAGAC-3'; 1/96.12) (SEQ ID NO 25) was used for reverse transcription and PCR and

35 synthesized from the 3' end of the PRRS ORF4 gene. The

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PCR product was digested with EcoRI and BamHI to yield a fragment approximately 542 base pairs in length corresponding to the PRRS ORF4 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction
5 fragment of plasmid pJF751 (25). Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

10 Homology vector 902-49.34

The plasmid 902-49.34 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine reproductive and respiratory syndrome virus (PRRS) ORF5
15 gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
20 GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PRRS ORF5 gene is under the control of the late/early promoter (LP2EP2). It was
25 constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction
30 fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an EcoRI to BamHI restriction

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fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the NVSL (Reference strain, IA-2). To synthesize PRRS ORF5, the primer (5'-
5 TTGAATTCGTTGGAGAAATGCTTGACCGCGGGC-3'; 1/96.13) (SEQ ID NO 26) synthesized from the 5' end of the PRRS ORF5 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-
GAAGGATCCTAAGGACGACCCCATTGTTCCGCTG-3'; 1/96.14) (SEQ ID
10 NO 27) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF5 gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 606 base pairs in length corresponding to the PRRS ORF5 gene. Fragment 3 is an
15 approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 4 were converted to unique NotI sites
20 using NotI linkers.

Homology vector 902-49.46

The plasmid 902-49.46 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by RPV
25 DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING
30 RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and a second foreign DNA is inserted into

an EcoRI or BamHI site, and the second foreign DNA is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 3 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers. Synthetic DNA between fragments 2, and 3 contains the LP2EP2 promoter and an EcoRI site and a BamHI site for insertion of foreign DNA. (See Figure 6).

20 Homology vector 902-67.1

The plasmid 902-67.1 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 1500 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 3600 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and a second foreign DNA is inserted into an EcoRI or BamHI site, and the second foreign DNA is

under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 2200 base pair HindIII to SnaBI restriction sub-fragment of the RPV HindIII restriction fragment N (6). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 3 is an approximately 2900 base pair SnaBI to HindIII subfragment of the RPV HindIII fragment N. The SnaB I sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers. Synthetic DNA between fragments 2 and 3 contains the LP2EP2 promoter and an EcoRI site and a BamHI site for insertion of foreign DNA. (See Figure 6).

Homology vector 902-67.14

The plasmid 902-67.14 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 1500 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 3600 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and a second foreign DNA is inserted into an EcoRI or BamHI site, and the second foreign DNA is under the control of the late/early promoter (LP2EP2).

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It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an
5 approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 1500 base pair HindIII to EcoRV restriction sub-fragment of the RPV HindIII restriction fragment N (6). Fragment 2 is an approximately 3010
10 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 3 is an approximately 3600 base pair EcoRV to HindIII subfragment of the RPV HindIII fragment N. The EcoRV sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.
15 Synthetic DNA between fragments 2 and 3 contains the LP2EP2 promoter and an EcoRI site and a BamHI site for insertion of foreign DNA. (See Figure 6).

Homology vector 902-67.27

The plasmid 902-67.27 was constructed for the purpose of
20 inserting foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 2200 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 2900 base pair
25 fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late
30 promoter (LP1) and a second foreign DNA is inserted into an EcoRI or BamHI site, and the second foreign DNA is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA

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techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 2200 base pair HindIII to EcoRV restriction sub-fragment of the RPV HindIII restriction fragment N (6). Fragment 2 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 3 is an approximately 2900 base pair EcoRV to HindIII subfragment of the RPV HindIII fragment N. The EcoRV sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers. Synthetic DNA between fragments 2 and 3 contains the LP2EP2 promoter and an EcoRI site and a BamHI site for insertion of foreign DNA. (See Figure 6).

Homology vector 919-16.12

The plasmid 919-16.12 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1) and a second foreign DNA is inserted into an EcoRI or BamHI site, and the foreign DNA is under the control of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments

from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an
5 approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an approximately 895 base pair XbaI to
10 HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers. Synthetic DNA between fragments 2 and 3 contains the LP2EP2 promoter and an EcoRI site and a BamHI site for insertion of foreign
15 DNA. (See Figure 5).

Homology vector 919-63.21

The plasmid 919-63.21 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene and a porcine
20 parvovirus (PPV) VP2 capsid protein gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the
25 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter (LP1) and the PPV VP2 gene is under the control
30 of the late/early promoter (LP2EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid

vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is a BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PPV obtained from the NADL (Strain NADL-2; non-pathogenic vaccine strain). To synthesize PPV VP2, the primer (5'-CTAAGGATCCGAGTGAAAATGTGGAACAACACAACCC-3'; 4/97.50) (SEQ ID NO 31) synthesized from the 5' end of the PPV VP2 gene, introduced an BamHI site at the 5' end of the gene. The primer (5'-GTGGATCCTAGTATAATTTTCTTGGTATAAGTTGTGAA -3'; 4/97.51) (SEQ ID NO 32) was used for reverse transcription and PCR and synthesized from the 3' end of the PPV VP2 gene. The PCR product was digested with BamHI to yield a fragment approximately 1700 base pairs in length corresponding to the PPV VP2 gene. Fragment 3 is an approximately 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 4 were converted to unique NotI sites using NotI linkers.

Homology vector 934-64.2

The plasmid 934-64.2 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 (Eichelberger strain) flanked by RPV DNA. Upstream of the foreign genes is an approximately

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906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1) and the PRRS ORF3 is inserted into an EcoRI and BamHI site, and is under the control of the early promoter (EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF3, the primer 5'-CAATGAATTCTAGCTGTGCACTCCTCCATATTTTCCTC-3'; 9/97.9) (SEQ ID NO 45) synthesized from the 5' end of the PRRS ORF3 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-CGGGAATTCCTATCGCCGTACGGCACTGAGG-3'; 9/97.10) (SEQ ID NO 46) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF3 gene, introduced an EcoRI site at the 3' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 768 base pairs in length corresponding to the PRRS ORF3 gene.

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Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

5 Homology vector 934-65.1

The plasmid 934-65.1 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF2 (Eichelberger strain) flanked by RPV DNA. Upstream of the foreign genes is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the

10 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1) and the PRRS ORF2 is inserted into an

15 EcoRI and BamHI site, and is under the control of the early promoter (EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was

20 derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base

25 pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from

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a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF2, the primer 5'-CTGAGAATTCATGGGGGCTATGCAAAGCCTTTTCG -3'; 9/97.13) (SEQ ID NO 43) synthesized from the 5' end of the PRRS ORF2 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'- GTGGAATTCACCGTGAGTTCGAAAGAAAAATTGC -3'; 9/97.14) (SEQ ID NO 44) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF2 gene, introduced an EcoRI site at the 3' end of the gene. The PCR product was digested with EcoRI to yield a fragment approximately 771 base pairs in length corresponding to the PRRS ORF2 gene. Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

Homology vector 935-50.1

The plasmid 935-50.1 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene flanked by RPV DNA. Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene' is under the control of a late promoter (LP1). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII

restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

10 Homology vector 936-87.2

The plasmid 936-87.2 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 (Eichelberger strain) flanked by RPV DNA. Upstream of the foreign genes is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the

20 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1) and the PRRS ORF7 is inserted into an

25 EcoRI and BamHI site, and is under the control of the early promoter (EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was

30 derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction

fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF 7, the primer 5' - TATGAATTCTAACAACGGCAAGCAGCAAAAGAAAAAG -3'; 9/97.15) (SEQ ID NO 53) synthesized from the 5' end of the PRRS ORF7 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'- TGTGGATCCATCACGCTGTGGGTGATGCTGTAG -3'; 9/97.16) (SEQ ID NO 54) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF7 gene, introduced an BamHI site at the 3' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 372 base pairs in length corresponding to the PRRS ORF7 gene.

Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

Homology vector 938-94.1

The plasmid 938-94.1 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF4 (Eichelberger strain) flanked by RPV DNA. Upstream of the foreign genes is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the

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HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1) and the PRRS ORF4 is inserted into an EcoRI and BamHI site, and is under the control of the early promoter (EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF4, the primer 5'- GGGAAATTCTGCGTCCCTTCTTTTCTCTTGGTTG -3'; 9/97.7) (SEQ ID NO 47) synthesized from the 5' end of the PRRS ORF4 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'- CTTGGATCCTCAAATTGCCAGCAGGATGGCAAAAAG -3'; 9/97.8) (SEQ ID NO 48) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF4 gene, introduced an BamHI site at the 3' end of the gene. The PCR product was digested with EcoRI to yield a fragment approximately 542 base pairs in length corresponding to the PRRS ORF4 gene.

Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The

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XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

Homology vector 938-94.25

The plasmid 938-94.25 was constructed for the purpose of inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 (Eichelberger strain) flanked by RPV DNA. Upstream of the foreign genes is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1) and the PRRS ORF5 is inserted into an EcoRI and BamHI site, and is under the control of the early promoter (EP2). It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS

ORF5, the primer 5'- CAAGGAATTCGGGGAAATGCTTGACCGCGGGCTG
-3'; 9/97.5) (SEQ ID NO 49) synthesized from the 5' end
of the PRRS ORF5 gene, introduced an EcoRI site at the
5' end of the gene. The primer (5'-
5 AAAAGGATCCTAGGGACGACCCCATTGTTCAGC -3'; 9/97.6) (SEQ ID
NO 50) was used for reverse transcription and PCR and
synthesized from the 3' end of the PRRS ORF5 gene,
introduced an BamHI site at the 3' end of the gene. The
PCR product was digested with EcoRI and BamHI to yield
10 a fragment approximately 606 base pairs in length
corresponding to the PRRS ORF5 gene.

Fragment 4 is an approximately 895 base pair XbaI to
HindIII subfragment of the RPV HindIII fragment U. The
XbaI sites in fragments 1 and 3 were converted to unique
15 NotI sites using NotI linkers.

Homology vector 950-13.4

The plasmid 950-13.4 was constructed for the purpose of
inserting foreign DNA into RPV. It incorporates an E.
coli β -glucuronidase (uidA) marker gene and the gene for
20 porcine reproductive and respiratory syndrome virus
(PRRS) ORF6 (Eichelberger strain) flanked by RPV DNA.
Upstream of the foreign genes is an approximately 906
base pair fragment of RPV DNA. Downstream of the foreign
genes is an approximately 895 base pair fragment of RPV
25 DNA. When the plasmid is used according to the
HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING
RECOMBINANT RPV, a virus containing DNA coding for the
foreign genes will result. Note that the β -glucuronidase
(uidA) marker gene is under the control of a late
30 promoter (LP1) and the PRRS ORF6 is inserted into an
EcoRI and BamHI site, and is under the control of the
early promoter (EP2). It was constructed utilizing

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standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF6, the primer 5' - CAATGAATTCGTCCTAGACGACTTTTGCAATGATAG -3'; 9/97.5) (SEQ ID NO 51) synthesized from the 5' end of the PRRS ORF6 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5' - CTTGGGATCCTTATTTGGCATATTTGACAAGGTTTACCAC -3'; 9/97.6) (SEQ ID NO 52) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF6 gene, introduced an BamHI site at the 3' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 525 base pairs in length corresponding to the PRRS ORF6 gene.

Fragment 4 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

Homology vector 950-16.34

The plasmid 950-16.34 was constructed for the purpose of

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inserting foreign DNA into RPV. It incorporates an E. coli β -glucuronidase (uidA) marker gene and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and ORF6 (Eichelberger strain) flanked by RPV DNA. Upstream of the foreign genes is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -glucuronidase (uidA) marker gene is under the control of a late promoter (LP1), the PRRS ORF5 is inserted into an EcoRI and BamHI site, and is under the control of the early promoter (EP2), and PRRS ORF6 is inserted into an EcoRI and BamHI site, and is under the control of the late promoter (LP2),. It was constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector was derived from an approximately 2999 base pair HindIII restriction fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an approximately 1823 base pair NotI restriction fragment of plasmid pRAJ260 (CLONETECHTM). Fragment 3 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF5, the primer 5'- CAAGGAATTTCGGGGAAATGCTTGACCGCGGGCTG -3'; 9/97.5) (SEQ ID NO 49) synthesized from the 5' end of the PRRS ORF5 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5'-

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AAAAGGATCCTAGGGACGAGCCCATTGTTTCAGC -3'; 9/97.6) (SEQ ID NO 50) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF5 gene, introduced an BamHI site at the 3' end of the gene. The
5 PCR product was digested with EcoRI and BamHI to yield a fragment approximately 606 base pairs in length corresponding to the PRRS ORF5 gene.

Fragment 4 is an EcoRI to BamHI restriction fragment synthesized by reverse transcription and polymerase
10 chain reaction (PCR) using genomic RNA from a U.S. Isolate of PRRS obtained from the Eichelberger atypical strain. To synthesize PRRS ORF6, the primer 5'-CAATGAATTCGTCCTAGACGACTTTTGCAATGATAG -3'; 9/97.5) (SEQ ID NO 51) synthesized from the 5' end of the PRRS ORF6
15 gene, introduced an EcoRI site at the 5' end of the gene. The primer (5' -CTTGGGATCCTTATTTGGCATATTTGACAAGGTTTACCAC -3'; 9/97.6) (SEQ ID NO 52) was used for reverse transcription and PCR and synthesized from the 3' end of the PRRS ORF6
20 gene, introduced an BamHI site at the 3' end of the gene. The PCR product was digested with EcoRI and BamHI to yield a fragment approximately 525 base pairs in length corresponding to the PRRS ORF6 gene.

Fragment 5 is an approximately 895 base pair XbaI to
25 HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

Homology vector for S-RPV-014

The plasmid is constructed for the purpose of inserting
30 foreign DNA into RPV. It incorporates an E. coli β -galactosidase (lacZ) marker gene flanked by RPV DNA.

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Upstream of the foreign gene is an approximately 906 base pair fragment of RPV DNA. Downstream of the foreign genes is an approximately 895 base pair fragment of RPV DNA. When the plasmid is used according to the

5 HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV, a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase (lacZ) marker gene is under the control of a late promoter, swinepox virus I5L (Figure 9). It is

10 constructed utilizing standard recombinant DNA techniques (20, 21), by joining restriction fragments from the following sources with the synthetic DNA sequences. The plasmid vector is derived from an approximately 2999 base pair HindIII restriction

15 fragment of pSP64 (PROMEGATM). Fragment 1 is an approximately 906 base pair HindIII to XbaI restriction sub-fragment of the RPV HindIII restriction fragment U (6). Fragment 2 is an 3010 base pair BamHI to PvuII restriction fragment of plasmid pJF751 (25). Fragment 3

20 is an approximately 895 base pair XbaI to HindIII subfragment of the RPV HindIII fragment U. The XbaI sites in fragments 1 and 3 were converted to unique NotI sites using NotI linkers.

EXAMPLE 1:

25 Recombinant raccoonpox virus (RPV) capable of replication which contains foreign DNA encoding an antigenic polypeptide is useful to prevent disease in porcine, bovine, sheep, goats, ovine, caprine, feline, canine, avian, equine, and human species. Useful

30 insertion sites for foreign DNA in non-essential regions in the raccoonpox virus genome are in genomic fragments HindIII U (2.1 kb), HindIII M (6.0 kb), HindIII N (5.1 kb), HindIII W (1.5 kb),, HindIII T (2.2 kb, HindIII P

(4.5 kb). Open reading frames in each of these fragments which are suitable for insertion of foreign DNA include, but are not limited to: O1L homolog, E11L homolog in HindIII U; D1L homolog, C17L/B23R homolog in HindIII M; 5 D1L homolog, B22R homolog in HindIII N; N1L homolog, N2L homolog, M1L homolog, M2L homolog, K1L homolog, K2L homolog, K7R homolog in HindIII W, T, or P.

EXAMPLE 2: S-RPV-001

10 S-RPV-001 is a raccoonpox virus that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) HA (H1N1) were inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ 15 gene is under the control of the synthetic late promoter (LP1), and the SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2). Transcription of the SIV HA gene is opposite to the direction of transcription of the lacZ gene and opposite to the 20 direction of transcription of the RPV O1L ORF.

S-RPV-001 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-16.2 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR 25 GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-001. This virus was assayed for 30 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial

three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-RPV-001 was assayed for expression of SIV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Polyclonal swine anti-SIV serum or polyclonal goat anti-HA serum was shown to react specifically with S-RPV-001 plaques and not with S-RPV-000 negative control plaques. All S-RPV-001
10 observed plaques reacted with the antiserum indicating that the virus was stably expressing the SIV HA protein.

To confirm the expression of the SIV HA protein gene product, cells were infected with S-RPV-001 and samples of infected cell lysates and culture supernatants were
15 subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal swine anti-SIV serum or polyclonal goat anti-HA serum was used to detect expression of SIV specific proteins. The cell lysate and culture
20 supernatant from S-RPV-001 infected cells exhibited bands corresponding to 64 kd, which is the expected size of the SIV HA protein.

S-RPV-001 is useful as a vaccine in swine against swine influenza infection. S-RPV-001 is useful as a vaccine in
25 combination with S-RPV-003 which expresses SIV NA. S-RPV-001 is also useful for expression of the SIV HA protein.

EXAMPLE 3: S-RPV-002

S-RPV-002 (ATCC Accession No. VR-2597) is a raccoonpox
30 virus (RPV) that expresses at least two foreign genes.

The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) HA (H1N1) were inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV HA gene is under the control of the synthetic late/early promoter (LP2EP2). In contrast to S-RPV-001, direction of transcription of the SIV HA gene in S-RPV-002 is the same as the direction of transcription of the lacZ gene and the direction of transcription of the RPV O1L ORF.

S-RPV-002 was deposited on February 6, 1998 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va 20108-0971, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

S-RPV-002 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-19.18 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-002. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-RPV-002 was assayed for expression of SIV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Polyclonal swine anti-SIV serum or polyclonal goat anti-HA serum was shown to
5 react specifically with S-RPV-002 plaques and not with S-RPV-000 negative control plaques. All S-RPV-002 observed plaques reacted with the antiserum indicating that the virus was stably expressing the SIV HA protein.

To confirm the expression of the SIV HA protein gene
10 product, cells were infected with S-RPV-002 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. Polyclonal swine anti-SIV serum or polyclonal
15 goat anti-HA serum was used to detect expression of SIV specific proteins. The cell lysate and culture supernatant from S-RPV-002 infected cells exhibited bands corresponding to 64 kd, which is the expected size of the SIV HA protein.

20 S-RPV-002 is useful as a vaccine in swine against swine influenza infection. S-RPV-002 is useful as a vaccine in combination with S-RPV-003 which expresses SIV NA. S-RPV-002 is also useful for expression of the SIV HA protein.

25 EXAMPLE 4: S-RPV-003

S-RPV-003 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for swine influenza virus (SIV) NA (H1N1) were inserted into the RPV O1L ORF
30 (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic

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fragment. The lacZ gene is under the control of the synthetic late promoter (LP1), and the SIV NA gene is under the control of the synthetic late/early promoter (LP2EP2).

5 S-RPV-003 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-49.5 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
10 GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-003. This virus was assayed for β -galactosidase expression, purity, and insert stability
15 by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

20 S-RPV-003 was assayed for expression of SIV-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Polyclonal swine anti-SIV serum was shown to react specifically with S-RPV-003 plaques and not with S-RPV-000 negative control plaques.
25 All S-RPV-003 observed plaques reacted with the antiserum indicating that the virus was stably expressing the SIV NA protein.

To confirm the expression of the SIV NA protein gene product, cells were infected with S-RPV-003 and samples
30 of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING

PROCEDURE. Polyclonal goat anti-NA serum was used to detect expression of SIV specific proteins. The cell lysate and culture supernatant from S-RPV-003 infected cells exhibited bands corresponding to 64 kd, which is
5 the expected size of the SIV NA protein.

S-RPV-003 is useful as a vaccine in swine against swine influenza infection. S-RPV-003 is useful as a vaccine in combination with S-RPV-001 or S-RPV-002 which express SIV HA. S-RPV-003 is also useful for expression of the
10 SIV NA protein.

Polyclonal murine antibodies to SIV NA were produced using S-RPV-003 according to the PROCEDURE TO PRODUCE MURINE POLYCLONAL ANTIBODIES TO PROTEINS EXPRESSED IN RECOMBINANT RACCOONPOX VIRUS. BLACK PLAQUE ASSAYS were
15 conducted, using the mouse anti S-RPV-003 serum, against recombinant swinepox virus (SPV) expressing swine influenza virus HA or NA proteins. The mouse anti S-RPV-003 serum (1:100 dil) was shown to react specifically with recombinant SPV expressing NA. The serum did not
20 cross react to either recombinant SPV expressing SIV HA or -galactosidase.

EXAMPLE 5: S-RPV-004

S-RPV-004 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine
25 reproductive and respiratory syndrome virus (PRRS) ORF3 were inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ gene is under the control of the synthetic late promoter
30 (LP1), and the PRRS ORF3 gene is under the control of

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the synthetic late/early promoter (LP2EP2).

5 S-RPV-004 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-49.14 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-004. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

20 S-RPV-004 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Polyclonal swine anti-PRRS serum was shown to react specifically with S-RPV-004 plaques and not with S-RPV-000 negative control plaques. All S-RPV-004 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF3 protein.

25 To confirm the expression of the PRRS ORF3 protein gene product, cells were infected with S-RPV-004 and samples of infected cell lysates and culture supernatants were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS serum was used to detect expression of PRRS specific proteins. The cell lysate and culture

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supernatant from S-RPV-004 infected cells exhibited a band corresponding to 45 kd, which is the expected size of the PRRS ORF3 protein. ORF3 protein is secreted from infected cells into the culture media.

5 S-RPV-004 is useful as a vaccine in swine against PRRS infection. S-RPV-004 is useful as a vaccine in combination with S-RPV-005 and S-RPV-006 which express PRRS ORF4 and PRRS ORF5, respectively. S-RPV-004 is also useful for expression of the PRRS ORF3 protein.

10 Polyclonal murine antibodies to PRRS ORF3 were produced using S-RPV-004 according to the PROCEDURE TO PRODUCE MURINE POLYCLONAL ANTIBODIES TO PROTEINS EXPRESSED IN RECOMBINANT RACCOONPOX VIRUS. BLACK PLAQUE ASSAYS are conducted, using the mouse anti S-

15 RPV-004 serum, against recombinant swinepox virus (SPV) expressing PRRS ORF3, ORF4 or ORF5 proteins.

EXAMPLE 6: S-RPV-005

S-RPV-005 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF4

20 were inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ

25 gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF4 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-RPV-005 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology

30 vector 902-49.23 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR

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GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-005. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-RPV-005 was assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Polyclonal swine anti-PRRS serum was shown to react specifically with S-RPV-005 plaques and not with S-RPV-000 negative control plaques. All S-RPV-005 observed plaques reacted with the antiserum indicating that the virus was stably expressing the PRRS ORF4 protein.

To confirm the expression of the PRRS ORF4 protein gene product, cells were infected with S-RPV-005 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A polyclonal swine anti-PRRS serum was used to detect expression of PRRS specific proteins. The cell lysate from S-RPV-005 infected cells exhibited a band corresponding to 31 kd, which is the expected size of the PRRS ORF4 protein (202 amino acids).

S-RPV-005 is useful as a vaccine in swine against PRRS infection. S-RPV-005 is useful as a vaccine in combination with S-RPV-004 and S-RPV-006 which express

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PRRS ORF3 and PRRS ORF5, respectively. S-RPV-005 is also useful for expression of the PRRS ORF4 protein.

Polyclonal murine antibodies to PRRS ORF4 were produced using S-RPV-005 according to the PROCEDURE TO
5 PRODUCE MURINE POLYCLONAL ANTIBODIES TO PROTEINS EXPRESSED IN RECOMBINANT RACCOONPOX VIRUS. BLACK PLAQUE ASSAYS are conducted, using the mouse anti S-RPV-005 serum, against recombinant swinepox virus (SPV) expressing PRRS ORF3, ORF4 or ORF5 proteins.

10 EXAMPLE 7: S-RPV-006

S-RPV-006 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5
15 were inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF5 gene is under the control of
20 the synthetic late/early promoter (LP2EP2).

S-RPV-006 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-49.34 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
25 GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-006. This virus was assayed for
30 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay

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as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 To confirm the expression of the PRRS ORF5 protein gene product, cells were infected with S-RPV-006 and samples of infected cell lysates were subjected to SDS polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. A
10 polyclonal swine anti-PRRS serum was used to detect expression of PRRS specific proteins. The cell lysate from S-RPV-006 infected cells exhibited a band corresponding to 26 kd, which is the expected size of the PRRS ORF5 protein.

15 S-RPV-006 is useful as a vaccine in swine against PRRS infection. S-RPV-006 is useful as a vaccine in combination with S-RPV-004 and S-RPV-005 which express PRRS ORF3 and PRRS ORF4, respectively. S-RPV-006 is also useful for expression of the PRRS ORF5 protein.

20 EXAMPLE 8: S-RPV-007

S-RPV-007 is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -galactosidase (lacZ) was inserted into the RPV OlL ORF (a unique NotI restriction site has replaced an XbaI
25 restriction site) within the RPV HindIII "U" genomic fragment. The lacZ gene is under the control of the synthetic late promoter (LP1), and second foreign DNA is inserted into a unique EcoRI or BamHI site under the control of the synthetic late/early promoter (LP2EP2).

30 S-RPV-007 was derived from S-RPV-000 (ATCC Strain VR-

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838). This was accomplished utilizing the homology vector 902-49.46 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-007. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-RPV-007 is useful as a vaccine in mammalian and avian species. When a foreign DNA encoding an antigenic polypeptide form an avian or mammalian pathogen, or encoding a cytokine or immune-modulating protein is inserted under the control of the LP2EP2 promoter in an EcoRI or BamHI site in S-RPV-007, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

The XbaI site within the RPV genomic fragment HindIII U is a useful insertion site for foreign DNA. A second foreign DNA is inserted into S-RPV-007 at a unique EcoRI or BamHI insertion site and is under control of the synthetic late/early promoter, LP2EP2. When the second foreign DNA encodes an antigenic polypeptide from an avian or mammalian pathogen, or encodes a cytokine or immune-modulating protein, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

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EXAMPLE 9: S-RPV-008

S-RPV-008 (ATTC Accession No. VR-2598) is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -galactosidase (lacZ) was inserted
5 into a unique SnaB I site within the RPV genomic fragment HindIII N (a unique NotI restriction site has replaced a SnaBI restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1), and second foreign DNA is inserted into a unique EcoRI
10 or BamHI site under the control of the synthetic late/early promoter (LP2EP2).

S-RPV-008 was deposited on February 6, 1998 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va 20108-0971, U.S.A.
15 under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

S-RPV-008 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology
20 vector 902-67.1 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final
25 result of red plaque purification was the recombinant virus designated S-RPV-008. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial
30 three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-RPV-008 is useful as a vaccine in mammalian and avian species. When a foreign DNA encoding an antigenic polypeptide from an avian or mammalian pathogen, or encoding a cytokine or immune-modulating protein is inserted under the control of the LP2EP2 promoter in an EcoRI or BamHI site in S-RPV-008, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

The SnaBI site within the RPV genomic fragment HindIII N is a useful insertion site for foreign DNA. A second foreign DNA is inserted into S-RPV-008 at a unique EcoRI or BamHI insertion site and is under control of the synthetic late/early promoter, LP2EP2. When the second foreign DNA encodes an antigenic polypeptide from an avian or mammalian pathogen, or encodes a cytokine or immune-modulating protein, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

EXAMPLE 10: S-RPV-009

S-RPV-009 (ATCC Accession No. VR-2599) is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -galactosidase (lacZ) was inserted into a unique EcoRV site within the RPV genomic fragment HindIII N (a unique NotI restriction site has replaced an EcoRV restriction site). The lacZ gene is under the control of the synthetic late promoter (LP1).

S-RPV-009 was deposited on February 6, 1998 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va 20108-0971, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of

Microorganisms for the Purposes of Patent Procedure.

S-RPV-009 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-67.14 (see Materials and Methods) and virus
5 S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant
10 virus designated S-RPV-009. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were
15 blue indicating that the virus was pure, stable, and expressing the foreign gene.

S-RPV-009 is useful as a vaccine in mammalian and avian species. When a foreign DNA encoding an antigenic polypeptide form an avian or mammalian pathogen, or
20 encoding a cytokine or immune-modulating protein is inserted under the control of the LP2EP2 promoter in an EcoRI or BamHI site in S-RPV-009, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

25 The EcoRV site within the RPV genomic fragment HindIII N is a useful insertion site for foreign DNA. A second foreign DNA is inserted into S-RPV-009 at a unique EcoRI or BamHI insertion site and is under control of the synthetic late/early promoter, LP2EP2. When the second
30 foreign DNA encodes an antigenic polypeptide from an avian or mammalian pathogen, or encodes a cytokine or immune-modulating protein, the resulting recombinant

virus is useful as a vaccine against the avian or mammalian pathogen.

EXAMPLE 11: S-RPV-010

5 S-RPV-010 is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -galactosidase (lacZ) was inserted into a unique HpaI site within the RPV genomic fragment HindIII M (a unique NotI restriction site has replaced a HpaI restriction site). The lacZ gene is under the control of the
10 synthetic late promoter (LP1).

S-RPV-010 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 902-67.27 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR
15 GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-010. This virus was assayed for
20 β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

S-RPV-010 is useful as a vaccine in mammalian and avian species. When a foreign DNA encoding an antigenic polypeptide form an avian or mammalian pathogen, or
25 encoding a cytokine or immune-modulating protein is inserted under the control of the LP2EP2 promoter in an EcoRI or BamHI site in S-RPV-010, the resulting recombinant virus is useful as a vaccine against the
30 avian or mammalian pathogen.

The HpaI site within the RPV genomic fragment HindIII M is a useful insertion site for foreign DNA. A second foreign DNA is inserted into S-RPV-010 at a unique EcoRI or BamHI insertion site and is under control of the synthetic late/early promoter, LP2EP2. When the second foreign DNA encodes an antigenic polypeptide from an avian or mammalian pathogen, or encodes a cytokine or immune-modulating protein, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

EXAMPLE 12: S-RPV-011

S-RPV-011 is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -glucuronidase (uidA) was inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1).

S-RPV-011 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 919-16.12 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification was the recombinant virus designated S-RPV-011. This virus was assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and

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expressing the foreign gene.

S-RPV-C11 is useful as a vaccine in mammalian and avian species. When a second foreign DNA encoding an antigenic polypeptide from an avian or mammalian pathogen, or encoding a cytokine or immune-modulating protein is inserted under the control of the LP2EP2 promoter in a unique EcoRI or BamHI site in S-RPV-011, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

10 EXAMPLE 13: S-RPV-012

S-RPV-012 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -galactosidase (lacZ) and the gene for porcine parvovirus (PPV) VP2 capsid protein were inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ gene is under the control of the synthetic late promoter (LP1), and the PPV VP2 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-RPV-012 was derived from S-RPV-000 (ATCC Strain VR-838). This was accomplished utilizing the homology vector 919-63.21 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -galactosidase (BLUOGALTM AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-RPV-012. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay

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as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable, and expressing the foreign gene.

5 S-RPV-012 is useful as a vaccine in swine against porcine parvovirus infection. S-RPV-012 is also useful for expression of the PPV VP2 protein.

EXAMPLE 14: S-RPV-013

10 S-RPV-013 is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -glucuronidase (uidA) is inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the
15 synthetic late promoter (LP1).

S-RPV-013 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR 935-50.1 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING
20 RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-013. This virus is assayed for β -
25 glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is pure, stable, and
30 expressing the foreign gene.

S-RPV-013 is useful as a vaccine in mammalian and avian species. When a second foreign DNA encoding an antigenic polypeptide from an avian or mammalian pathogen, or encoding a cytokine or immune-modulating protein is inserted under the control of a promoter in a unique site in S-RPV-013, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen.

EXAMPLE 15: S-RPV-014

S-RPV-014 is a raccoonpox virus (RPV) that expresses at least one foreign gene. The gene for E. coli β -galactosidase (lacZ) is inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The lacZ gene is under the control of the swinepox virus I5L promoter (see Figure 9).

S-RPV-014 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR FOR S-RPV-014 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -GALACTOSIDASE (BLUOGALTM ASSAY). The final result of blue plaque purification is the recombinant virus designated S-RPV-014. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is expressing the foreign gene.

S-RPV-014 is useful as a vaccine in mammalian and avian species. When a second foreign DNA encoding an antigenic polypeptide from an avian or mammalian pathogen, or encoding a cytokine or immune-modulating protein is inserted under the control of a promoter in a unique site in S-RPV-014, the resulting recombinant virus is useful as a vaccine against the avian or mammalian pathogen. The SPV I5L promoter is a useful heterologous promoter in a recombinant raccoonpox virus. THE SPV I5L promoter is a strong late promoter.

EXAMPLE 16: S-RPV-015

S-RPV-015 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -glucuronidase (uidA) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF2 (Eichelberger strain) are inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF2 gene is under the control of the synthetic early promoter (EP2).

S-RPV-015 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR 934-65.1 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-015. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay

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as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

5 S-RPV-015 is assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Rabbit polyclonal anti β -glucuronidase antibody is shown to react specifically with S-RPV-015 plaques and not with S-RPV-000 negative
10 control plaques.

S-RPV-015 is useful as a vaccine in swine against PRRS infection. S-RPV-015 is useful as a vaccine in combination with S-RPV-016, S-RPV-017, S-RPV-018, S-RPV-019, and S-RPV-020 which express PRRS ORF3, ORF4, ORF5,
15 ORF6 and ORF7, respectively. S-RPV-015 is also useful for expression of the PRRS ORF2 protein for purposes of raising antibodies to PRRS ORF2 and for diagnostic assays.

EXAMPLE 17: S-RPV-016

20 S-RPV-016 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -glucuronidase (uidA) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF3 (Eichelberger strain) are inserted into the RPV O1L ORF
25 (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF3 gene is under the control of the synthetic late/early promoter
30 (LP2EP2).

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S-RPV-016 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR 934-64.2 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-016. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-RPV-016 is assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Rabbit polyclonal anti β -glucuronidase antibody is shown to react specifically with S-RPV-016 plaques and not with S-RPV-000 negative control plaques.

S-RPV-016 is useful as a vaccine in swine against PRRS infection. S-RPV-016 is useful as a vaccine in combination with S-RPV-015, S-RPV-017, S-RPV-018, S-RPV-019, and S-RPV-020 which express PRRS ORF2, ORF4, ORF5, ORF6 and ORF7, respectively. S-RPV-016 is also useful for expression of the PRRS ORF3 protein for purposes of raising antibodies to PRRS ORF3 and for diagnostic assays.

30 EXAMPLE 18: S-RPV-017

S-RPV-017 is a raccoonpox virus (RPV) that expresses at

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least two foreign genes. The gene for E. coli β -glucuronidase (uidA) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF4 (Eichelberger strain) are inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF4 gene is under the control of the synthetic late/early promoter (LP2EP2).

S-RPV-017 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR 938-94.1 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-017. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-RPV-017 is assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Rabbit polyclonal anti β -glucuronidase antibody is shown to react specifically with S-RPV-017 plaques and not with S-RPV-000 negative control plaques.

S-RPV-017 is useful as a vaccine in swine against PRRS

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infection. S-RPV-017 is useful as a vaccine in combination with S-RPV-015, S-RPV-016, S-RPV-018, S-RPV-019, and S-RPV-020 which express PRRS ORF2, ORF3, ORF5, ORF6 and ORF7, respectively. S-RPV-017 is also useful
5 for expression of the PRRS ORF4 protein for purposes of raising antibodies to PRRS ORF4 and for diagnostic assays.

EXAMPLE 19: S-RPV-018

S-RPV-018 is a raccoonpox virus (RPV) that expresses at
10 least two foreign genes. The gene for E. coli β -glucuronidase (uidA) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 (Eichelberger strain) are inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI
15 restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF5 gene is under the control of the synthetic early promoter (EP2).

S-RPV-018 is derived from S-RPV-000 (ATCC Strain VR-
20 838). This is accomplished utilizing the HOMOLOGY VECTOR 938-94.25 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -
25 glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-018. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay
30 as described in Materials and Methods. After the initial

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three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

5 S-RPV-018 is assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Rabbit polyclonal anti β -glucuronidase antibody is shown to react specifically with S-RPV-018 plaques and not with S-RPV-000 negative control plaques.

10 S-RPV-018 is useful as a vaccine in swine against PRRS infection. S-RPV-018 is useful as a vaccine in combination with S-RPV-015, S-RPV-016, S-RPV-017, S-RPV-019, and S-RPV-020 which express PRRS ORF2, ORF3, ORF4, ORF6 and ORF7, respectively. S-RPV-018 is also useful
15 for expression of the PRRS ORF5 protein for purposes of raising antibodies to PRRS ORF5 and for diagnostic assays.

EXAMPLE 20: S-RPV-019

20 S-RPV-019 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -glucuronidase (uidA) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF6 (Eichelberger strain) are inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI
25 restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF6 gene is under the control of the synthetic late promoter (LP2).

S-RPV-019 is derived from S-RPV-000 (ATCC Strain VR-
30 838). This is accomplished utilizing the HOMOLOGY VECTOR

950-13.4 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-019. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed are blue indicating that the virus is expressing the foreign gene.

S-RPV-019 is useful as a vaccine in swine against PRRS infection. S-RPV-019 is useful as a vaccine in combination with S-RPV-015, S-RPV-016, S-RPV-017, S-RPV-018, and S-RPV-020 which express PRRS ORF2, ORF3, ORF4, ORF5 and ORF7, respectively. S-RPV-019 is also useful for expression of the PRRS ORF6 protein for purposes of raising antibodies to PRRS ORF6 and for diagnostic assays.

EXAMPLE 21: S-RPV-020

S-RPV-020 is a raccoonpox virus (RPV) that expresses at least two foreign genes. The gene for E. coli β -glucuronidase (uidA) and the gene for porcine reproductive and respiratory syndrome virus (PRRS) ORF7 (Eichelberger strain) are inserted into the RPV O1L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), and the PRRS ORF7 gene is under the control of the synthetic early promoter (EP2).

S-RPV-020 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR 936-87.2 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-020. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-RPV-020 is assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Rabbit polyclonal anti β -glucuronidase antibody is shown to react specifically with S-RPV-020 plaques and not with S-RPV-000 negative control plaques.

S-RPV-020 is useful as a vaccine in swine against PRRS infection. S-RPV-020 is useful as a vaccine in combination with S-RPV-015, S-RPV-016, S-RPV-017, S-RPV-018, and S-RPV-019 which express PRRS ORF2, ORF3, ORF4, ORF5 and ORF6, respectively. S-RPV-020 is also useful for expression of the PRRS ORF7 protein for purposes of raising antibodies to PRRS ORF7 and for diagnostic assays.

EXAMPLE 22: S-RPV-028

S-RPV-028 is a raccoonpox virus (RPV) that expresses at

least three foreign genes. The gene for E. coli β -glucuronidase (uidA) and the genes for porcine reproductive and respiratory syndrome virus (PRRS) ORF5 and ORF6 (Eichelberger strain) are inserted into the RPV 01L ORF (a unique NotI restriction site has replaced an XbaI restriction site) within the RPV HindIII "U" genomic fragment. The uidA gene is under the control of the synthetic late promoter (LP1), the PRRS ORF5 gene is under the control of the synthetic early promoter (EP2) and the PRRS ORF6 gene is under the control of the synthetic late promoter (LP1).

S-RPV-028 is derived from S-RPV-000 (ATCC Strain VR-838). This is accomplished utilizing the HOMOLOGY VECTOR 950-16.34 (see Materials and Methods) and virus S-RPV-000 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV. The transfection stock is screened by the SCREEN FOR RECOMBINANT RPV EXPRESSING β -glucuronidase (X-GLUCTM ASSAYS). The final result of blue plaque purification is the recombinant virus designated S-RPV-028. This virus is assayed for β -glucuronidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus is pure, stable, and expressing the foreign gene.

S-RPV-028 is assayed for expression of PRRS-specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT RPV. Rabbit polyclonal anti β -glucuronidase antibody is shown to react specifically with S-RPV-020 plaques and not with S-RPV-000 negative control plaques.

S-RPV-028 is useful as a vaccine in swine against PRRS infection. S-RPV-028 is useful as a vaccine in combination with S-RPV-015, S-RPV-016, S-RPV-017, which express PRRS ORF2, ORF3, and ORF4, respectively. S-RPV-028 is also useful for expression of the PRRS ORF5 and ORF6 proteins for purposes of raising antibodies to PRRS ORF5 and ORF6 and for diagnostic assays.

EXAMPLE 23: Expression of the RPV K1L or C7L genes in recombinant swinepox virus to broaden the host range of recombinant swinepox virus.

The K1L gene in vaccinia virus determines the range of replication of the virus in the animal host. Deletion of this gene in vaccinia virus restricts viral replication in the host animal. Sequencing of the near terminal region of the swinepox virus genome indicates that the K1L gene is not present in this region and may be absent in the swinepox virus genome. (26). The K1L homologue in raccoonpox virus is located within the HindIII "T" RPV genomic fragment (SEQ ID NO. 5 and 10). The host range of raccoonpox is broader than swinepox virus (which replicates mainly in swine); Raccoonpox host range includes raccoons, dogs, cats, swine, cattle, monkeys and other mammals (See Table 1). A recombinant swinepox virus comprising the RPV K1L ORF under the control of the synthetic pox promoter, K1L promoter or LP2EP2 (or LP1 or LP2 or EP1 or EP2), has a broader host range to include raccoons, dogs, cats, and other mammals. Preferably, the promoter is the K1L promoter from raccoonpox which will express the K1L protein at the correct stage of the viral life cycle. A modified host range recombinant swinepox virus, which also expresses foreign DNA coding for an antigen from a disease pathogen, is an improved vaccine in raccoons, dogs,

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cats, and other mammals since it will replicate in the animals, expressing the foreign DNA and elicit a strong immune response against the antigen from the disease pathogen. The modified host range recombinant swinepox virus does not cause disease in the vaccinated animal.

In an alternative embodiment, recombinant swinepox virus comprising the RPV C7L ORF (host range gene) under the control of the synthetic pox promoter, C7L promoter or LP2EP2 (or LP1 or LP2 or EP1 or EP2), has a broader host range to include raccoons, dogs, cats, and other mammals. Preferably, the promoter is the C7L promoter from raccoonpox which will express the C7L protein at the correct stage of the viral life cycle. A modified host range recombinant swinepox virus, which also expresses foreign DNA coding for an antigen from a disease pathogen, is an improved vaccine in raccoons, dogs, cats, and other mammals since it will replicate in the animals, expressing the foreign DNA and elicit a strong immune response against the antigen from the disease pathogen. The modified host range recombinant swinepox virus does not cause disease in the vaccinated animal.

S-SPV-248:

S-SPV-248 is a swinepox virus (SPV) that expresses at least two foreign genes. The gene for raccoonpox virus K1L host range is inserted into a unique NotI restriction site located distal to the E. Coli β -galactosidase (lacZ) gene contained in a plasmid homology vector which deletes the swinepox virus I4L and I5L genes. The lacZ gene is under the control of the swinepox I5L and the K1L gene is under control of the raccoonpox virus K1L promoter.

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SPV-248 is derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock is
5 screened using the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification is the recombinant virus designated S-SPV-248. This virus is assayed for β -
10 galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods.

S-SPV-248 is useful as a vaccine against disease in mammals. S-SPV-248 has a broader host range than other
15 recombinant SPV due to expression of the RPV K1L gene. The host range includes, but is not limited to, raccoons, dogs, cats, horses, cows, sheep, goats, and other mammals. S-SPV-248 is also useful as a vaccine in avian species.

20 S-SPV-249:

S-SPV-249 is a swinepox virus (SPV) that expresses at least two foreign genes. The gene for raccoonpox virus C7L host range is inserted into a unique NotI
25 restriction site located distal to the E. Coli β -galactosidase (lacZ) gene contained in a plasmid homology vector which deletes the swinepox virus I4L and I5L genes. The lacZ gene is under the control of the swinepox I5L promoter and the C7L gene is under the control of the raccoonpox virus C7L promoter.

30 SPV-249 is derived from S-SPV-001 (Kasza Strain). This was accomplished utilizing a homology vector and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING SPV. The transfection stock is screened

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using the SCREEN FOR RECOMBINANT SPV EXPRESSING β -galactosidase (SCREEN FOR RECOMBINANT HERPESVIRUS EXPRESSING ENZYMATIC MARKER GENES). The final result of blue plaque purification is the recombinant virus designated S-SPV-249. This virus is assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Material and Methods.

S-SPV-249 is useful as a vaccine against disease in mammals. S-SPV-249 has a broader host range than other recombinant SPV due to expression of the RPV C7L gene. The host range includes, but is not limited to, raccoons, dogs, cats, horses, cows, sheep,, goats and other mammals. S-SPV-249 is also useful as a vaccine in avian species.

Deletion of host range genes from recombinant raccoonpox virus:

In a further embodiment, deletion of the host range genes selected from the group consisting of C7L, C6L, C5L, C4L, C3L, C2L, C1L, N1L, N2L, M1L, M2L, and K1L ("C7L to K1L") in a raccoonpox virus vector results in limited replication of the recombinant raccoonpox in Vero cells and no replication in other cell types, such as CRFK or MDCK cells. The recombinant raccoonpox with a deletion of some or all of the C7L to K1L host range genes is useful as a vaccine which is safe and less virulent than recombinant raccoonpox containing the C7L to K1L genes. To select for recombinant raccoonpox expressing a foreign DNA of interest, a homology vector containing some or all of the host range genes C7L to K1L and a foreign gene of interest under the control of a promoter is combined with recombinant raccoonpox virus deleted for the C7L to K1L genes in the HOMOLOGOUS

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RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT RPV
and selected for growth in cell types other than Vero
cells, such as CRFK or MDCK cells. Any recombinant virus
which grows in the other cell types must contain the
5 host range genes C7L to K1L and the foreign gene of
interest.

TABLE 1:

Virus	ESK-4 (Porcine)	PKWRL (Porcine)	VERO (Monkey)	CRFK (Feline)	MDCK (Canine)	MDBK (Bovine)
Raccoon pox virus	+	+	+++	+++	+++	+/-
Swinepox virus	+++	+++	+++	-	-	-
Vaccinia virus	+++	ND	+++	+++	+++	+++
Swinepox virus with RcnPV K1L gene	+++	+++	+++	+++	+++	+/-

+++ Extensive CPE

- No CPE

ND Not Determined

EXAMPLE 24: Nonessential regions in RPV genomic fragments W, T, and P for insertion of foreign DNA into a recombinant raccoonpox virus.

Nonessential insertion regions in RPV genomic fragments W, T, and P are useful for constructing recombinant raccoonpox viruses expressing foreign DNA which is inserted into a genomic region of the recombinant RPV. RPV genomic fragments W, T, and P contain open reading frames for vaccinia virus homologues N1L, N2L, M1L, M2L, K1L, K2L, and K7R. (SEQ ID NO. 5 -13) (17) These are all nonessential genes and are useful for insertion of foreign DNA to produce a recombinant raccoonpox virus. Intergenic regions between the ORFs are also useful for

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insertion of foreign DNA. Some of the restriction sites which are useful for insertion of foreign DNA in this region are shown in figure 2. Preferred insertion sites for foreign DNA in the HindIII W genomic fragment are Bcl I, Acc I, Pvu II, and Xba I; Preferred insertion sites for foreign DNA in the HindIII T genomic fragment are EcoR I, BamH I, Sal I, Bgl II, Pst I, Acc I, Pvu II, Xho I, and Xba I; Preferred insertion sites for foreign DNA in the HindIII P genomic fragment are Bcl I, Acc I, Xmn I and Xba I.

N1L is a virulence gene (virokine); K1L is a host range gene. It is important for use in swinepox virus vector to broaden the host range of SPV; K2L is a serine protease inhibitor (SPI-3); K3L is a translation initiation factor; confers interferon resistance; K4L is a member of the phospholipase D superfamily.

EXAMPLE 25: Nonessential regions in RPV genomic fragment S for insertion of foreign DNA into a recombinant raccoonpox virus.

Nonessential insertion regions in RPV genomic fragment S are useful for constructing recombinant raccoonpox viruses expressing foreign DNA which is inserted into a genomic region of the recombinant RPV. RPV genomic fragment S contains open reading frames for vaccinia virus homologues B19R, B20R and variola B18R. (SEQ ID NO. 33 to 35) (27). Amino acids 7 to 523 of SEQ ID NO 35 has homology to variola virus B18R ORF. Amino acids 235 to 399 has homology to vaccinia virus B20R ORF. These are all nonessential genes and are useful for insertion of

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foreign DNA to produce a recombinant raccoonpox virus. Intergenic regions between the ORFs are also useful for insertion of foreign DNA. Some of the restriction sites which are useful for insertion of foreign DNA in this region are shown in figure 8. Preferred insertion sites for foreign DNA in the HindIII S genomic fragment are Bgl II, EcoR I, Pme I, Spe I, Sal I, Acc I, BamH I, Bcl I, and Nde I.

Vaccinia B19R is a surface antigen present on infected cells. Vaccinia B20R and Variola B18R are homologous to neuraxin protein and map 1B protein (25).

EXAMPLE 26: Mapping of raccoonpox virus genomic fragments HindIII M and HindIII R

Restriction endonuclease mapping and DNA sequencing of the raccoonpox virus genome indicates differences in the HindIII restriction map compared to the published HindIII restriction map. In the present invention, the size of the HindIII M genomic region of RPV is 6.0 kb. The previously published size of the HindIII M genomic region of RPV is 5.4 kb (5). In the present invention, the HindIII J and HindIII W genomic fragments of RPV are immediately adjacent. An earlier published HindIII restriction map of the RPV genome shows HindIII R genomic fragment between HindIII J and HindIII W genomic fragments (6).

EXAMPLE 27: Raccoonpox virus EllL Promoter

The raccoonpox virus EllL promoter is useful as a homologous promoter in raccoonpox or a heterologous promoter in other poxviruses for the expression of

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foreign DNA in a recombinant poxvirus (Figure 7; SEQ ID NO: 30). Other poxviruses include, but are not limited to, swinepox, fowlpox, avipox, canarypox, cowpox and vaccinia virus.

5 EXAMPLE 28: Swinepox virus I5L Promoter

10 The swinepox virus (SPV) I5L promoter is useful as a homologous promoter in swinepox or a heterologous promoter in other poxviruses for the expression of foreign DNA in a recombinant poxvirus (Figure 9; SEQ ID NO: 55). Other poxviruses include, but are not limited to, raccoonpox, fowlpox, avipox, canarypox, cowpox and vaccinia virus. The SPV I5L promoter is contained within the SPV HindIII N genomic fragment. The SPV I5L promoter is a strong late promoter.

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What is claimed is:

1. A recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "U" genomic region of the raccoonpox region of the raccoonpox virus genome.
5
2. The recombinant raccoonpox virus of claim 1, wherein the foreign DNA sequence is inserted into an O1L open reading frame.
10
3. The recombinant raccoonpox virus of claim 1, wherein the foreign DNA sequence is inserted into an E11L open reading frame.
4. The recombinant raccoonpox virus of claim 1, wherein the foreign DNA sequence is inserted into an intergenic region.
15
5. The recombinant raccoonpox virus of claim 2, wherein the foreign DNA sequence is inserted into an XbaI restriction endonuclease site.
20
6. The recombinant raccoonpox virus of claim 1, which further comprises the foreign DNA sequence inserted into the HindIII "U" genomic region, wherein the foreign DNA is capable of being expressed in a recombinant raccoonpox virus host cell.
25
7. The recombinant raccoonpox virus of claim 1, wherein the foreign DNA sequence encodes a screenable marker.

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8. The recombinant raccoonpox virus of claim 7,
wherein the screenable marker is *E. coli*
beta-galactosidase.
9. The recombinant raccoonpox virus of claim 7,
5 wherein the screenable marker is *E. coli*
beta-glucuronidase.
10. The recombinant raccoonpox virus of claim 1,
wherein the foreign DNA sequence encodes an
antigenic polypeptide.
- 10 11. The recombinant raccoonpox virus of claims
10, wherein the antigenic polypeptide when
introduced into the host cell, induces
production of protective antibodies against a
feline disease-causing agent from which the
15 antigenic polypeptide is derived or
derivable.
12. The recombinant raccoonpox virus of claim 11,
wherein the antigenic polypeptide is derived
20 or derivable from a group consisting of
feline pathogen, canine pathogen, equine
pathogen, bovine pathogen, caprine pathogen,
avian pathogen, porcine pathogen, or human
pathogen.
- 25 13. The recombinant raccoonpox virus of claim 1,
wherein the foreign DNA sequence is selected
from a group consisting of swine influenza
virus hemagglutinin, swine influenza virus
neuraminidase, porcine reproductive and
30 respiratory virus (PRRS) ORF2, PRRS ORF3,
PRRS ORF4, PRRS ORF5, PRRS ORF6, PRRS ORF7,

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porcine parvovirus VP2.

14. The recombinant raccoonpox virus of claim 1,
wherein the foreign gene is under control of
an endogenous upstream, raccoonpox virus
promoter.
15. The recombinant raccoonpox virus of claim 14,
wherein promoter is selected from a group
consisting of the raccoonpox virus E11L
promoter and the raccoonpox virus O1L
promoter.
16. The recombinant raccoonpox virus of claim 1,
wherein the foreign gene is under control of
a heterologous upstream promoter.
17. The recombinant raccoonpox virus of claim 16,
wherein promoter is selected from a group
consisting of the synthetic pox late promoter
1 (LP1), the synthetic pox early promoter 1
(EP1), the synthetic pox early promoter 2
(EP2), the synthetic pox late promoter 2
early promoter 2 (LP2EP2), the synthetic pox
late promoter 1 (LP1), the synthetic pox late
promoter 2 (LP2), and the swinepox virus I5L
promoter.
18. The recombinant raccoonpox virus of claim 1
designated S-RPV-001.
19. The recombinant raccoonpox virus of claim 1
designated S-RPV-002.
20. The recombinant raccoonpox virus of claim 1

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designated S-RPV-003.

21. The recombinant raccoonpox virus of claim 1
designated S-RPV-004.
22. The recombinant raccoonpox virus of claim 1
5 designated S-RPV-005.
23. The recombinant raccoonpox virus of claim 1
designated S-RPV-006.
24. The recombinant raccoonpox virus of claim 1
designated S-RPV-007.
- 10 25. The recombinant raccoonpox virus of claim 1
designated S-RPV-011.
26. The recombinant raccoonpox virus of claim 1
designated S-RPV-012.
- 15 27. The recombinant raccoonpox virus of claim 1
designated S-RPV-013.
28. The recombinant raccoonpox virus of claim 1
designated S-RPV-015.
29. The recombinant raccoonpox virus of claim 1
designated S-RPV-016.
- 20 30. The recombinant raccoonpox virus of claim 1
designated S-RPV-017.
31. The recombinant raccoonpox virus of claim 1
designated S-RPV-018.

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32. The recombinant raccoonpox virus of claim 1 designated S-RPV-019.
33. The recombinant raccoonpox virus of claim 1 designated S-RPV-020.
- 5 34. The recombinant raccoonpox virus of claim 1 designated S-RPV-028.
- 10 35. A recombinant swinepox virus comprising a swinepox virus genome which contains a foreign DNA sequence inserted into a non-essential region of the raccoonpox virus genome, wherein the foreign DNA sequence is a host range gene selected from the group consisting of raccoonpox virus K1L and raccoonpox virus C7L.
- 15 36. The recombinant swinepox virus of claim 35, further comprising a second foreign DNA sequence inserted into a non-essential region within the swinepox virus genome.
- 20 37. The recombinant swinepox virus of claim 36 designated S-SPV-248.
38. The recombinant swinepox virus of claim 36 designated S-SPV-249.
- 25 39. A recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "N" genomic region of the raccoonpox virus genome.

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40. The recombinant raccoonpox virus of claim 39, wherein the foreign DNA sequence is inserted into an EcoRI restriction endonuclease site.
- 5 41. The recombinant raccoonpox virus of claim 39, wherein the foreign DNA sequence is inserted into an SnaBI restriction endonuclease site.
42. The recombinant raccoonpox virus of claim 40 designated S-RPV-008.
- 10 43. The recombinant raccoonpox virus of claim 41 designated S-RPV-009.
44. A recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a foreign DNA sequence inserted into a non-essential region within the HindIII "M" genomic region of the raccoonpox virus genome.
- 15 45. The recombinant raccoonpox virus of claim 44, wherein the foreign DNA sequence is inserted into an HpaI restriction endonuclease site.
46. The recombinant virus of claim 44 designated S-RPV-010.
- 20 47. A recombinant raccoonpox virus comprising a raccoonpox virus viral genome which contains a deletion in a raccoonpox virus host range gene of the viral genome, wherein the raccoonpox virus host range gene is selected from the group consisting of C7L, C6L, C5L, C4L, C3L, C2L, C1L, N1L, N2L, M1L, M2L, and K1L.
- 25 48. The recombinant raccoonpox virus of claim 47, further comprising a second foreign DNA sequence

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inserted into a non-essential region within the raccoonpox virus genome.

49. A homology vector for producing a recombinant raccoonpox virus by inserting a foreign DNA sequence into the raccoonpox virus genome which comprises a double-stranded DNA molecule consisting of:
- 5
- a) double-stranded foreign DNA sequence encoding an antigenic polypeptide derived from an animal pathogen;
- 10
- b) at one end of the foreign DNA sequence, double-stranded feline virus genomic DNA homologous to the genomic DNA located at one side of a non-essential site of the raccoonpox viral genomic DNA;
- 15
- c) at the other end of the foreign DNA sequence, double-stranded raccoonpox virus genomic DNA homologous to the genomic DNA located at the other side of the same site.
50. The homology vector of claim 49, wherein the antigenic polypeptide is selected from a group consisting of swine influenza virus hemagglutinin, swine influenza virus neuraminidase, porcine reproductive and respiratory virus (PRRS) ORF2, PRRS ORF3, PRRS, ORF4, PRRS ORF5, PRRS ORF6, PRRS ORF7, porcine parvovirus VP2, *E. coli* beta-galactosidase gene., *E. Coli* beta-glucuronidase gene.
- 20
- 25
51. The homology vector claim 49, designated Homology Vector 902-16.2.
52. The homology vector of claim 49, designated Homology Vector 902-19.18.
- 30

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53. The homology vector of claim 49, designated Homology Vector 902-49.5.
54. The homology vector of claim 49, designated Homology Vector 902-49.14.
- 5 55. The homology vector of claim 49, designated Homology Vector 902-49.23.
56. The homology vector of claim 49, designated Homology Vector 902-49.34.
- 10 57. The homology vector of claim 49, designated Homology Vector 934.65-1.
58. The homology vector of claim 49, designated Homology Vector 934-64.2.
59. The homology vector of claim 49, designated Homology Vector 938-94.1.
- 15 60. The homology vector of claim 49, designated Homology Vector 938-94.25.
61. The homology vector of claim 49, designated Homology Vector 950-13.4.
- 20 62. The homology vector of claim 49, designated Homology Vector 936-87.2.
63. The homology vector of claim 49, designated Homology Vector 950-16.34.
64. A host cell infected with the recombinant raccoonpox virus of claim 1.
- 25 65. A host cell of claim 64, wherein the host cell is

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mammalian cell.

- 5 66. A vaccine against an animal pathogen which comprises an effective immunizing amount of the recombinant raccoonpox virus of claim 1 and a suitable carrier.
67. A method of immunizing an animal against an animal pathogen which comprises administering to the animal an effective immunizing dose of the vaccine of claim 66.
- 10 68. A method of immunizing an animal against a feline pathogen which comprises administering to the animal an effective immunizing dose of the vaccine of claim 66.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Schering-Plough Ltd.
- (ii) TITLE OF INVENTION: Recombinant Raccoonpox virus
- (iii) NUMBER OF SEQUENCES: 58
- (iv) CORRESPONDENCE ADDRESS:
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 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: USA
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- (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:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: White, John P.
 - (B) REGISTRATION NO: 28,678
 - (C) REFERENCE/DOCKET NUMBER: 55744-pct
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2061 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Raccoonpox virus ATCC# VR-838

(1x) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 386..1

(1x) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2061..372

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAGCTTAATC CATCATAAAA TTTATGGTTA GTAACGACTG AAATAACTGC GTAATCATT	60
TTTGGTACAA TTCTAGTCGG CATAGATTCT GTAACAATGA ATTCGATTCC CGTTACTTTA	120
GTAAAATCTT TTACAAATAA CAAGGATTCA TCAAATACGT AAAACTCATT ATTTACTATA	180
GAAATTGATC CCCTATCACA CTTGAAATAA AAAATATCTT TATCCTTTAA CACCAAATAA	240
AATTCAGATT GGTCAATATG AATGTATTCA CTTAGTAATT CAACGAACTT ATTTATTAAT	300
TCTGATACAC ACACTGATTC TGTATTGTTT ATCGAAAAC T TACTCTTCC TGTATCAGTT	360
TCTAAAAAAA TATTAACAAG TTCCATTTAT ATCATCCAAT ATTATTGAAA TGGTGGTAAT	420
AGACAAATGA TATAGGTAAG ACGGCACAGT ACCTTTGTCTG ACCATCTCCT CCATTTTCATG	480
CTCTATCTTA TCATGAACTT TGATATGTGA AAACAATACA CCACATGCTT CTATAACAGT	540
ATGTAACACT TTAGATACAA AATGCTTGAT ACCATCGTAA TTGTTCAACA CGGCCAATCT	600
ATAATAGATA GTAGCTACTA TATATTCTAT GATAGTATTG AAGAAGATAA CCACCTTGGC	660
ATATTGGTCA TTTAATACAG ACATGGTATC AACAGATAGC TTAAACGATA GGGAAATCCGT	720
GAATGGAATA AGCGTTTCAT TGATTGAATA TCCATATACT AACATATCGG ATATCCTGAT	780
GTGTCCATT AAATCGTTAA GTTTTTTCTT TTTAACCTCG TTGAACATCA TTTCTGTAA	840
CGGTCCCCAA CATCTTTGAC CAATTAATTT TTGATTTATT TTTCCATGTA TGGCGTATCT	900
AGTCAGGTCG TATAACCTAT CCAATAATCC ATCATCGGTA TCCAAATTAC ATACTATGCT	960
TTTCAATTCT CTATAGAAAA GATTAATACA TCTAGAACAA CAACATGATG TAATATCTTT	1020
ATCATCAACA GATGTGATAT ATTTGAAGAT TTTTCTATGA TAATGAAGAA TTTTGTAGAA	1080
TACTGTATTA ATGGTGCTG TTACCATGAT CCCTTTGATT GCTGACAGAG TCAGCGAGCA	1140
TGATTCCCAA TCTTTAACAA TTTTATTAC CATTATCTTT GTTTTAATGT CTATAGATGA	1200
TAACATAACA CGTCTAACAA TACACGGATT AAGACGAAA GATGAAATTA TTCTTTCAAC	1260
ATCTCCAATG GATAACTTAC TATTTGACG TGCATTGTCT ATATGCTCGA GAACATCCTC	1320
CAACGAGTCT GTATCTTTTT TGATTATCGT CGATCTCAAC GATTTGGGTC GTCTAGATCG	1380
TCGGACTCTA TCACCAGATG GCATAGTTAT TAGTCTTCTT TCTGTTTTCA TAATGAAATT	1440

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TCTAAATTCA TCTGCGAATC TTCTATATCT AAAATCATAA TATGCGATAT TTGTTTCAAC      1500
AAATTTCCGT TCGTCCAATG TCAACATATC TATCTCGGTT TCATACCCTA AATTGAACAT      1560
GGCTACGGAT TTAATTTTAT ATTCATCTAT CAACTCTTCT TCAACAATAA CAGAGTATAG      1620
ATAATCATTT AATCCATCAT ACATAGTAGG AAGATTCTCG TTAACAAATT GTTTAACTGC      1680
ACTGATAAAA CTGGGACTAT ATTTGATATC TTGTCTAATA AAATTAATAA CATTGTCCAA      1740
AGGATACTTT TTAAC TAGAT TTATACATAT CTGTTTCATCA GTGAGGTTAT GCAAAACATC      1800
GTGAATAGGT GGCACATTAT ATTCATCAGA TATACTAATA ACAATTTCCA GATCTATATT      1860
GTTTAATATA TTATATAGAT GGAGCACGGA TCCAACGGGG ATCTCTTTAA CCACATCACG      1920
GATTTTCATCA ACTGTTAAAT CTATTTTAAA GTTAATCATG TACGCGTTAA TTTTAAAG      1980
ATGTGTGGCT CTAAC TATAT TCTCACGAAT TAGCCATTCC AAGTCACTAC GTGAGAGAAG      2040
ATCGTATTCT ATCATAAGCT T                                     2061

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

```

Met Glu Leu Val Asn Ile Phe Leu Glu Thr Asp Thr Gly Arg Val Lys
1           5           10           15
Phe Ser Ile Asn Asn Thr Glu Ser Val Cys Val Ser Glu Leu Ile Asn
20           25           30
Lys Phe Val Glu Leu Leu Ser Glu Tyr Ile His Ile Asp Gln Ser Glu
35           40           45
Phe Tyr Leu Val Leu Lys Asp Lys Asp Ile Phe Tyr Phe Lys Cys Asp
50           55           60
Arg Gly Ser Ile Ser Ile Val Asn Asn Glu Phe Tyr Val Phe Asp Glu
65           70           75           80
Ser Leu Leu Phe Val Lys Asp Phe Thr Lys Val Thr Gly Ile Glu Phe
85           90           95

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Ile Val Thr Glu Ser Met Pro Thr Arg Ile Val Pro Lys Asn Asp Tyr
          100                      105                      110

Ala Val Ile Ser Val Val Thr Asn His Lys Phe Tyr Asp Gly Leu Ser
          115                      120                      125

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 688 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

```

Trp Pro Cys Ser Ser Phe Lys Asx Met Gln Lys Asp Glx Leu Asp His
1          5          10          15

Met Pro Ser Thr Cys Glx Val Gln Lys Lys Xaa Val Glu Pro Pro Phe
          20          25          30

Trp Leu Gln Tyr Asp Pro Gln Lys His Thr Phe Trp Trp Pro Glu His
          35          40          45

Thr Glx Leu Ala Ser Thr Gln Glu Ser His Ala His Val Ser Val Lys
          50          55          60

Xaa Cys Cys Thr Trp His Thr Asp Asn Ser Ala Asn Tyr His Gly Phe
          65          70          75          80

His Glx Glx Arg Ser Ile Val Asx Glu Xaa Asx Pro Trp His Pro Leu
          85          90          95

Ala Ser Glu Arg Glu Thr Pro Leu Ser His Pro Pro Leu Ser Pro Arg
          100          105          110

Ser Gln Arg Xaa Cys Arg Ile Glu Arg Xaa Leu Cys Arg Xaa Lys Leu
          115          120          125

Met Ile Glu Tyr Asp Leu Leu Ser Arg Ser Asp Leu Glu Trp Leu Ile
          130          135          140

Arg Glu Asn Ile Val Arg Ala Thr His Leu Leu Lys Ile Asn Ala Tyr
          145          150          155          160

Met Ile Asn Phe Lys Ile Asp Leu Thr Val Asp Glu Ile Arg Asp Val
          165          170          175

Val Lys Glu Ile Pro Val Gly Ser Val Leu His Leu Tyr Asn Ile Leu

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180										185										190										
Asn	Asn	Ile	Asp	Leu	Glu	Ile	Val	Ile	Ser	Ile	Ser	Asp	Glu	Tyr	Asn															
		195						200					205																	
Val	Pro	Pro	Ile	His	Asp	Val	Leu	His	Asn	Leu	Thr	Asp	Glu	Gln	Ile															
		210						215					220																	
Cys	Ile	Asn	Leu	Val	Lys	Lys	Tyr	Pro	Leu	Asp	Asn	Val	Ile	Asn	Phe															
		225						230					235		240															
Ile	Arg	Gln	Asp	Ile	Lys	Tyr	Ser	Pro	Ser	Phe	Ile	Ser	Ala	Val	Lys															
								245					250		255															
Gln	Phe	Val	Asn	Glu	Asn	Leu	Pro	Thr	Met	Tyr	Asp	Gly	Leu	Asn	Asp															
								260					265		270															
Tyr	Leu	Tyr	Ser	Val	Ile	Val	Glu	Glu	Glu	Leu	Ile	Asp	Glu	Tyr	Lys															
								275					280		285															
Ile	Lys	Ser	Val	Ala	Met	Phe	Asn	Leu	Gly	Tyr	Glu	Thr	Glu	Ile	Asp															
								290					295		300															
Met	Leu	Thr	Leu	Asp	Glu	Arg	Lys	Phe	Val	Glu	Thr	Asn	Ile	Ala	Tyr															
								305					310		315															
Tyr	Asp	Phe	Arg	Tyr	Arg	Arg	Phe	Ala	Asp	Glu	Phe	Arg	Asn	Phe	Ile															
								320					325		330															
Met	Lys	Thr	Glu	Arg	Arg	Leu	Ile	Thr	Met	Pro	Ser	Gly	Asp	Arg	Val															
								335					340		345															
Arg	Arg	Ser	Arg	Arg	Pro	Lys	Ser	Leu	Arg	Ser	Thr	Ile	Ile	Lys	Lys															
								350					355		360															
Asp	Thr	Asp	Ser	Leu	Glu	Asp	Val	Leu	Glu	His	Ile	Asp	Asn	Ala	Arg															
								365					370		375															
Arg	Asn	Ser	Lys	Leu	Ser	Ile	Gly	Asp	Val	Glu	Arg	Ile	Ile	Ser	Ser															
								380					385		390															
Phe	Arg	Leu	Asn	Pro	Cys	Ile	Val	Arg	Arg	Val	Met	Leu	Ser	Ser	Ile															
								395					400		405															
Asp	Ile	Lys	Thr	Lys	Ile	Met	Val	Ile	Lys	Ile	Val	Lys	Asp	Trp	Lys															
								410					415		420															
Ser	Cys	Ser	Leu	Thr	Leu	Ser	Ala	Ile	Lys	Gly	Ile	Met	Val	Thr	Asp															
								425					430		435															
Thr	Ile	Asn	Thr	Val	Phe	Ser	Lys	Ile	Leu	His	Tyr	His	Arg	Lys	Ile															
								440					445		450															
Phe	Lys	Tyr	Ile	Thr	Ser	Val	Asp	Asp	Lys	Asp	Ile	Thr	Ser	Cys	Cys															
								455					460		465															
Cys	Ser	Arg	Cys	Ile	Asn	Leu	Phe	Tyr	Arg	Glu	Leu	Lys	Ser	Ile	Val															
								470					475		480															
								485					490		495															

Cys Asn Leu Asp Thr Asp Asp Gly Leu Leu Asp Arg Leu Tyr Asp Leu
 500 505 510
 Thr Arg Tyr Ala Ile His Gly Lys Ile Asn Gln Lys Leu Ile Gly Gln
 515 520 525
 Arg Cys Trp Gly Pro Leu Thr Glu Met Met Phe Asn Glu Val Lys Lys
 530 535 540
 Lys Lys Leu Asn Asp Leu Met Glu His Ile Arg Ile Ser Asp Met Leu
 545 550 555 560
 Val Tyr Gly Tyr Ser Ile Asn Glu Thr Leu Ile Pro Phe Thr Asp Ser
 565 570 575
 Leu Ser Phe Lys Leu Ser Val Asp Thr Met Ser Val Leu Asn Asp Gln
 580 585 590
 Tyr Ala Lys Val Val Ile Phe Phe Asn Thr Ile Ile Glu Tyr Ile Val
 595 600 605
 Ala Thr Ile Tyr Tyr Arg Leu Ala Val Leu Asn Asn Tyr Asp Gly Ile
 610 615 620
 Lys His Phe Val Ser Lys Val Leu His Thr Val Ile Glu Ala Cys Gly
 625 630 635 640
 Val Leu Phe Ser His Ile Lys Val His Asp Lys Ile Glu His Glu Met
 645 650 655
 Glu Glu Met Val Asp Lys Gly Thr Val Pro Ser Tyr Leu Tyr His Leu
 660 665 670
 Ser Ile Thr Thr Ile Ser Ile Ile Leu Asp Asp Ile Asn Gly Thr Cys
 675 680 685

(2) INFORMATION FOR SEQ ID NO:4:

i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1793 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Raccoonpox virus ATCC# VR-838

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

AAGCTTCCCG TTACTTTAGT AAAATCTTTT ACAAATAACA AGGATTCATC AAATACGTAA 60
 AACTCATTAT TTACTATAGA AATTGATCCC CTATCACACT TGAAATAAAA AATATCTTTA 120

TCCTTTAACA CCAAATAAAA TTCAGATTGG TCAATATGAA TGTATTCACT TAGTAATTCA	180
ACGAACTTAT TTATTAATTC TGATACACAC ACTGATTCTG TATTGTTTAT CGAAAACTTT	240
ACTCTTCCTG TATCAGTTTC TAAAAAATA TTAACAAGTT CCATTTATAT CATCCAATAT	300
TATTGAAATG GTGGTAATAG ACAAATGATA TAGGTAAGAC GGCACAGTAC CTTTGTCTGAC	360
CATCTCCTCC ATTTTCATGCT CTATCTTATC ATGAACTTTG ATATGTGAAA ACAATACACC	420
ACATGCTTCT ATAACAGTAT GTAACACTTT AGATACAAAA TGCTTGATAC CATCGTAATT	480
GTTCAACACG GCCAATCTAT AATAGATAGT AGCTACTATA TATTCTATGA TAGTATTGAA	540
GAAGATAACC ACCTTGGCAT ATTGGTCATT TAATACAGAC ATGGTATCAA CAGATAGCTT	600
AAACGATAGG GAATCCGTGA ATGGAATAAG CGTTTCATTG ATTGAATATC CATATACTAA	660
CATATCGGAT ATCCTGATGT GTTCCATTAA ATCGTTAAGT TTTTCTTTT TAACCTCGTT	720
GAACATCATT TCTGTTAACG GTCCCCAACA TCTTTGACCA ATTAATTTTT GATTATTTTT	780
TCCATGTATG GCGTATCTAG TCAGGTCGTA TAACCTATCC AATAATCCAT CATCGGTATC	840
CAAATTACAT ACTATGCTTT TCAATTCTCT ATAGAAAAGA TTAATACATC TAGAACAACA	900
ACATGATGTA ATATCTTTAT CATCAACAGA TGTGATATAT TTGAAGATTT TTCTATGATA	960
ATGAAGAATT TTTGAGAATA CTGTATTAAT GGTGTCTGTT ACCATGATCC CTTTGATTGC	1020
TGACAGAGTC AGCGAGCATG ATTTCCAATC TTTAACAATT TTTATTACCA TTATCTTTGT	1080
TTTAATGTCT ATAGATGATA ACATAACACG TCTAACAATA CACGGATTAA GACGGAAAGA	1140
TGAAATTATT CTTTCAACAT CTCCAATGGA TAACCTACTA TTTGACGTG CATTGTCTAT	1200
ATGCTCGAGA ACATCCTCCA ACGAGTCTGT ATCTTTTTTG ATTATCGTCG ATCTCAACGA	1260
TTTGGGTCGT CTAGATCGTC GGA CTCTATC ACCAGATGGC ATAGTTATTA GTCTTCTTTC	1320
TGTTTTTATA ATGAAATTTT TAAATTCATC TGCGAATCTT CTATATCTAA AATCATAATA	1380
TGCGATATTT GTTTCAACAA ATTTCCGTTC GTCCAATGTC AACATATCTA TCTCGGTTTC	1440
ATACCCTAAA TTGAACATGG CTACGGATTT AATTTTATAT TCATCTATCA ACTCTCTTC	1500
AACAATAACA GAGTATAGAT AATCATTTAA TCCATCATAC ATAGTAGGAA GATTCTCGTT	1560
AACAAATTGT TTAACGTCAC TGATAAAACT GGGACTATAT TTGATATCTT GTCTAATAAA	1620
ATTAATAACA TTGTCCAAAG GATACTTTTT AACTAGATTT ATACATATCT GTTCATCAGT	1680
GAGGTTATGC AAAACATCGT GAATAGGTGG CACATTATAT TCATCAGATA TACTAATAAC	1740
AATTTCCAGA TCTATATTGT TTAATATATT ATATAGATGG AGCACGGAAG CTT	1793

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4491 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Raccoonpox virus ATCC# VR-838

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 81..1

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 855..181

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2152..912

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 2850..2194

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 3816..2950

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 4491..3970

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAGCTTTT	TA	AAATGATCAC	TGTAATATGA	TGAATCTGTA	TCATTTCTCC	AAATAATGTA	60
TCTGAATAGG	AGATTTCTCA	TATCTACTAA	TGTATTTAGT	TAGTAAACTA	TTATTGTGTT		120
AAATAAGTAT	TTATGAATTA	GATAACAATT	TTTAAAAGAC	TATGTTTGAT	ACAAAAAAAA		180
TTAATTTTGG	AAATACTTGT	CCCTCAAGTA	ATCGGCAAAG	CAATCTATAT	AATCATATGT		240
GACATTATCA	TATAATACAT	GAATATATAC	TGGATCGTAA	CTAGTTAAGA	TATACACGAA		300
TCCGATAACC	TTTTTTTACAT	AATCAACAGG	ATCTATTTTA	AATGTATCGT	ATCTAGTTCTG		360
CTTTATTTTT	CTGAATAGAT	TAATGTTGTA	ATTAGGATTT	CTATGTATGT	CTTTTATGGT		420
GTATTCCACG	TAGCTACCAT	AATGATCAAT	AGCCAATTGT	TTGTAAGTCA	TTAGCTCTTT		480

TATTATGTTA CATTGGTGT TACGTAGTTC ATCGCTGTTT ATTGCCAAGA ATCTATCTAC	540
AATTTTAAAT ACTTCAACGC CTGGATTATT AACCTGTTTC ATATACTGGG CTATATAATT	600
ATCAATATAT CGAGATGATA CAGCACCGTC GTACATTACA TACACATCTT CTCCTGAGTT	660
ATTCTGTGCT GATGGGCAGT TTAATACCTC CAGCATCTTG GTAACCTTAT TAGTATCCAT	720
TTTTTTAATA TCATCGAGTA ACACGGCTGA AGAATCACCA TCCATTACGA TAGCTATAAA	780
CACTAATCTA TATATTATTT TTATGTTATT TTTAAAGATC ATCAAAAATC TCATACGATA	840
CTCCTTCATG GACATAATTT GTTACAAAAT GTTTATATGT AGTAAAGTAT CTGCGATTTT	900
TGCAAAAATC TGGACGCATA AGACCAAAGT ATGCCAGGAA TGGAAATAATA ATAGATTTAT	960
CGAGTTTATC CTTTTCTACT GATTTTCATTG TTAGATACAT GGTCTCGGAA GTCGGATTAT	1020
GCAACATCAG TTTCGATAAT ATAACCTGGAT TATTTAACCT GACACATTCG CTCATACAAG	1080
TATGCCCAT TACAACCTGAG TCTACGCTAA AATGATTAAA CAATAGATAG TCTATCATCG	1140
TTTCGTATTC GAACAGAACA GCGTAGTACA TGGCATCTTC ACAAATTATA TCATTATCCA	1200
ATAGATATTT AACACATCTT ATAGATCCTG ACTTAACAGC TGTTTTAAAA TCATTAGAAT	1260
CAAATTGTCTG TCCTTTATCA TTAATACTTT CTAGAATGTC ACATCTATCG TAAATGATAC	1320
ACAGATTAAC TAGTTGATCT GTTATAGAAT CACTAGTTGA TATCAATTTG TTAAAGAGAG	1380
ATACAGGAGT TAATGTTTTA ATGAGAATGG TAAGAGGACT ATCGCCGAAC TTGTTTTGTT	1440
TATTAACATC TGTGATGGA AGCAAAAGAT CGATTATATC TATATTCTTG ACTGCTTTAG	1500
AACATGCAAT ATGGATAGGT GTATTTCCGT CATAATCTGC TCTTGAGGAA CTAATACCGA	1560
GTTTCATCAT CCACTCAATT GTACAAGCTT TTGGATTCTC AGACATAAGA TGTCTATGAA	1620
TATGATTTCT GCCAACTTTA TCTACTATCT TGGCCTCGAA TCCTATAGAC ATTAGTTTTT	1680
TGAACACTAT TTCTGACGGA TCTGTGCATG ATAGTAATGG TCCACATCCT TCTACATCAA	1740
CTGAGTTATT AATCTTAGCT CCGTGTTCCA CTAATAAATT TATTTTATCA ATTACATCAT	1800
CCTCGTTTCC GGAGAGATAA TACAGTGGAG TCTTATTTTG TTTATCGCAC GCGTTTGGAT	1860
TTGCACCATG CGTCAATAGC GTTGATATAA TTCTATTATT ATTGATTCTA GACGCTATAT	1920
GTAATGGATA ATCACCCTCA TCATTTGCTT CATTAGGCGA GTAGCCTTTA TTGATAAGTT	1980
CTTCTATGAA CTGCTCGTCT ATTCCTTTAA TTCCACAATA GATGTGTAAA ATACTATATT	2040
TTCCAGATGG TATTGTTGAC TGCAATATAT TTCTAAATAC ATCTAAATTC TTATGTTTTG	2100
ATTTGGCGTA AAGCGTTAGA TAATACTCGC TGTGAGGCAT GGTATCGTCC ATTGTTTCAT	2160
AAAAATGATA TTTCAATTTT TACTTTCTAC TTTTACTCT CTATAACAAA TGTCGTAAC	2220
AACATTTTTT ATGTCAAGAA AGCAACTGTT TAGTTCATCT TTGAATGTTA CGCCATAACT	2280

ACCTTGTGAA GCGTAATCGG AATTCCGATA AAGAAATTTA TCAACTGGAT GAACAAACTT	2340
TGGATTAATA TTAATAGGAT CCATGCGGAG GTCGACACAT TTGACGCATG ATCCATTGAT	2400
AGTTATAGCA CATTCTGTTT CATGATTCAT TGCTTCTGTA AGTTCTGAAT CGTATTTTGT	2460
AGTTCCACAA TTCATTTTCGG TACATGTTAT GGTTACACTA ATATTGTGCT GAAGTTTATC	2520
TAGTCGTTGA GTAGTAAACA ACAGAACTGA TAGTTTATAA TCTTCACCTA CTCCCTCTGC	2580
AGCTGCGACA AATCTCTGAT CCGTATCATA TATGGTCATA TTTATTTGTA ATCCATATCC	2640
TGTCAAACCT ATGTTTGCAT CTCTACCAGT GTAATTTTCA TACATGTGAC ACTCACCCAT	2700
AATAGTTTTG ATATCATAAT TAACACCAAT AGTGAGTTCG GCGGCAAAGT ACCAATAACG	2760
ATAATCACTT CGAGACGGAC ATTGCGTGTC TTTGTATTCT GAATAACCAA GAGATATGAC	2820
ACAAAAGAGT AAGAATAATC TGTAACCAT CTTTAAAGAC TGCAACAGAT CTACTAGACA	2880
AGAGTCAATG ATAGAAACCA CTACTAAAAA AAATAATTAT TCTATCAATT TTAACCAATT	2940
GATCTTATTT TAAAATTGGG ATCTAAATAT TTTTGCAGAA TTGACATACA TGATTCTGAG	3000
TTCTTATTT TTGCTAATTA TCTCATCCAA TTTATTATTC TTGACAACAT CGAGTTCTTT	3060
TATTAAATCA TCAGTCTTAT AGTCAACATG TTTTCTATA ATCATTCTAG CTATTACAGG	3120
ATCATCCAAC AATACATTTT CCAGATTAAC AGAATAGATA TTAATGTCAT ATTTGAACAG	3180
AGTCCGTAGC AAATCTAAGT CTTTGTTCTC TATAGCCAAT TTGATGTCTG GGATGAAGAG	3240
AAGAGAGTTA TTGGTATTTG TTGCCATCAT ATAGTCTAGT AACAGAATCA GCATATCAAC	3300
ATTTCCATTT TTAATTGTGT CATGAATGCA ACTATAGAGA ATTGCCAGAT CAAAACTAGC	3360
TTTtagctct GAAATAAAGT ATTCAGTAAT GCTGACATCA TTAAGCATAA CCGCCTTATA	3420
AAATGGGGTT TTCCATCCGG TTTTTCATA GAACATCAGT TTCCATTTTT TCTTAATAAA	3480
CAGTTTTATA GTTTGGATAT TGCCGGCCTC CACGGCGTAA TACAATGGAG TATTACCTCG	3540
ATCATCAAAT TGCGAATCAT CCATCCCACT GAATAACAAA ATCTTTACTA TTTTAGTATC	3600
CTCTAACTTG GCTGCCTGAT GCAATGGAAA CTCATTCTCG AGAAGATTTT TCAATACCCC	3660
AGCATTAAGT AATTCACAAA CAAGACGCAC GTTATTATTA TCAATTGCAT AATACAACGC	3720
CGTACGTCCA TGAATATCAG CCTTAAATAA GTCCTTTCCG CCGATGAAGC TTTTCAACTG	3780
CTTAGACTTC CAGGTATTAA TTCGTGACAG ATCCATGTCT GAAACGAGAC GCTAAATACA	3840
GTGTATGTTT CATTTTTTAT AATTTTGCAT TACATCAATC CTCCAGAGTC AATAATATCT	3900
CTGATCGATG TGATCAATAG ATAAATGGCT ATCGCAAAAC AACACAACCA CATTTAATAA	3960
AAATAATATT CAAGGAGATT CAACCTTACC AATAAATAAT ATAAATCCAG TAATATCATG	4020
TCGGATGATA AACACAAATG GTTTATTGAA CTCTAGTTCT ACAGGTGCCG ATCTTCCTGT	4080

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AGCCAACATT ATAGTAGACG CCTCTGCTAC TGTTCCTTGT TCATCAACAT CAATTTTAGC      4140
ATTCTGAAAC ATTTTATAGA TATATAATGG ATACTTGGTC ATGCGTTTAA ATGATGCATT      4200
GTCTGGATTA AACATACTGG GTGCAATCAT TTCGGCTATA GTCTTAATAT CTCTCTTATT      4260
TTCGATAGAA AATCTAGGAA GACTAAGATC ATACATTTTA TCCATTAATT GAGATGACCA      4320
ATAATCTAAT TTTGTGGTTG TGATAGAGTC CACAAAATGC GTGATATTAT CTCCAATTGC      4380
TATGTACATG CTAACATTAC TACCTTTATA TGGAAGTCTT ACCATGTCAT ATTCATAGTC      4440
ATCTACAGTA ATTGTGTTTC CTTGTAATTT AGCAACTAAA TTCATTGTTG G                4491

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

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Met Val Ile Leu Gln Pro Cys Tyr Ser Met Ile Leu Lys Lys Trp Ile
1           5              10              15

Leu Ile Lys Leu Pro Arg Cys Trp Arg Tyr
          20              25

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 224 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

```

Met Ser Met Lys Glu Tyr Arg Met Lys Phe Leu Met Ile Phe Lys Asn
1           5              10              15

Asn Ile Lys Ile Ile Tyr Arg Leu Val Phe Ile Ala Ile Val Met Asp
          20              25              30

Gly Asp Ser Ser Ala Val Leu Leu Asp Asp Ile Lys Lys Met Asp Thr

```

35	40	45
Asn Lys Val Thr Lys Met Leu Glu Val Leu Asn Cys Pro Ser Ala Gln		
50	55	60
Asn Asn Ser Gly Glu Asp Val Tyr Val Met Tyr Asp Gly Ala Val Ser		
65	70	75
Ser Arg Tyr Ile Asp Asn Tyr Ile Ala Gln Tyr Met Lys Gln Val Asn		
85	90	95
Asn Pro Gly Val Glu Val Phe Lys Ile Val Asp Arg Phe Leu Ala Met		
100	105	110
Asn Ser Asp Glu Leu Arg Asn Thr Lys Cys Asn Ile Ile Lys Glu Leu		
115	120	125
Met Thr Tyr Lys Gln Leu Ala Ile Asp His Tyr Gly Ser Tyr Val Glu		
130	135	140
Tyr Thr Ile Lys Asp Ile His Arg Asn Pro Asn Tyr Asn Ile Asn Leu		
145	150	155
Phe Arg Lys Ile Lys Arg Thr Arg Tyr Asp Thr Phe Lys Ile Asp Pro		
165	170	175
Val Asp Tyr Val Lys Lys Val Ile Gly Phe Val Tyr Ile Leu Thr Ser		
180	185	190
Tyr Asp Pro Val Tyr Ile His Val Leu Tyr Asp Asn Val Thr Tyr Asp		
195	200	205
Tyr Ile Asp Cys Phe Ala Asp Tyr Leu Arg Asp Lys Tyr Phe Gln Asn		
210	215	220

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 446 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

Met Asp Asp Thr Met Pro Asp Ser Glu Tyr Tyr Leu Thr Leu Tyr Ala		
1	5	10
Lys Ser Lys His Lys Asn Leu Asp Val Phe Arg Asn Ile Leu Gln Ser		
20	25	30
Thr Ile Pro Ser Gly Lys Tyr Ser Ile Leu His Ile Tyr Cys Gly Ile		
35	40	45
Lys Gly Ile Asp Glu Gln Phe Ile Glu Glu Leu Ile Asn Lys Gly Tyr		

50	55	60
Ser Pro Asn Glu Ala Asn Asp Asp Gly Asp Tyr Pro Leu His Ile Ala		
65	70	75 80
Ser Arg Ile Asn Asn Asn Arg Ile Ile Ser Thr Leu Leu Thr His Gly		
	85	90 95
Ala Asn Pro Asn Ala Cys Asp Lys Gln Asn Lys Thr Pro Leu Tyr Tyr		
	100	105 110
Leu Ser Gly Asn Glu Asp Asp Val Ile Asp Lys Ile Asn Leu Leu Val		
	115	120 125
Glu His Gly Ala Lys Ile Asn Asn Ser Val Asp Val Glu Gly Cys Gly		
	130	135 140
Pro Leu Leu Ser Cys Thr Asp Pro Ser Glu Ile Val Phe Lys Lys Leu		
	145	150 155 160
Met Ser Ile Gly Phe Glu Ala Lys Ile Val Asp Lys Val Arg Gly Asn		
	165	170 175
His Ile His Arg His Leu Met Ser Glu Asn Pro Lys Ala Cys Thr Ile		
	180	185 190
Glu Trp Met Met Lys Leu Gly Ile Ser Ser Ser Arg Ala Asp Tyr Asp		
	195	200 205
Gly Asn Thr Pro Ile His Ile Ala Cys Ser Lys Ala Val Lys Asn Ile		
	210	215 220
Asp Ile Ile Asp Leu Leu Leu Pro Ser Thr Asp Val Asn Lys Gln Asn		
	225	230 235 240
Lys Phe Gly Asp Ser Pro Leu Thr Ile Leu Ile Lys Thr Lys Thr Pro		
	245	250 255
Val Ser Leu Phe Asn Lys Leu Ile Ser Thr Ser Asp Ser Ile Thr Asp		
	260	265 270
Gln Leu Val Asn Leu Cys Ile Ile Tyr Asp Arg Cys Asp Ile Leu Glu		
	275	280 285
Ser Ile Asn Asp Lys Gly Arg Gln Phe Asp Ser Asn Asp Phe Lys Thr		
	290	295 300
Ala Val Lys Ser Gly Ser Ile Arg Cys Val Lys Tyr Leu Leu Asp Asn		
	305	310 315 320
Asp Ile Ile Cys Glu Asp Ala Met Tyr Tyr Ala Val Leu Phe Glu Tyr		
	325	330 335
Glu Thr Met Ile Asp Tyr Leu Leu Phe Asn His Phe Ser Val Asp Ser		
	340	345 350
Val Val Asn Gly His Thr Cys Met Ser Glu Cys Val Arg Leu Asn Asn		
	355	360 365

```

Pro Val Ile Leu Ser Lys Leu Met Leu His Asn Pro Thr Ser Glu Thr
  370                      375                      380

Met Tyr Leu Thr Met Lys Ser Val Glu Lys Asp Lys Leu Asp Lys Ser
  385                      390                      395                      400

Ile Ile Ile Pro Phe Leu Ala Tyr Phe Gly Leu Met Arg Pro Asp Phe
                      405                      410                      415

Cys Lys Asn Arg Arg Tyr Phe Thr Thr Tyr Lys His Phe Val Thr Asn
                      420                      425                      430

Tyr Val His Glu Gly Val Ser Tyr Glu Val Phe Asp Asp Leu
  435                      440                      445

```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 218 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

```

Met Val Tyr Arg Leu Phe Leu Leu Phe Cys Val Ile Ser Leu Gly Tyr
  1                      5                      10                      15

Ser Glu Tyr Lys Asp Thr Gln Cys Pro Ser Arg Ser Asp Tyr Arg Tyr
                      20                      25                      30

Trp Tyr Phe Ala Ala Glu Leu Thr Ile Gly Val Asn Tyr Asp Ile Lys
                      35                      40                      45

Thr Ile Met Gly Glu Cys His Met Tyr Glu Asn Tyr Thr Gly Arg Asp
                      50                      55                      60

Ala Asn Ile Gly Leu Thr Gly Tyr Gly Leu Gln Ile Asn Met Thr Ile
  65                      70                      75                      80

Tyr Asp Thr Asp Gln Arg Phe Val Ala Ala Ala Glu Gly Val Gly Glu
                      85                      90                      95

Asp Tyr Lys Leu Ser Val Leu Leu Phe Thr Thr Gln Arg Leu Asp Lys
                      100                     105                     110

Leu Gln His Asn Ile Ser Val Thr Ile Thr Cys Thr Glu Met Asn Cys
                      115                     120                     125

Gly Thr Thr Lys Tyr Asp Ser Glu Leu Thr Glu Ala Met Asn His Glu
                      130                     135                     140

Thr Glu Cys Ala Ile Thr Ile Asn Gly Ser Cys Val Lys Cys Val Asp
  145                     150                     155                     160

```

Leu Arg Met Asp Pro Ile Asn Ile Asn Pro Lys Phe Val His Pro Val
 165 170 175

Asp Lys Phe Leu Tyr Arg Asn Ser Asp Tyr Ala Ser Gln Gly Ser Tyr
 180 185 190

Gly Val Thr Phe Lys Asp Glu Leu Asn Ser Cys Phe Leu Asp Ile Lys
 195 200 205

Asn Val Ser Tyr Asp Ile Cys Tyr Arg Glu
 210 215

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 288 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

Met Asp Leu Ser Arg Ile Asn Thr Trp Lys Ser Lys Gln Leu Lys Ser
 1 5 10 15

Phe Ile Gly Gly Lys Asp Leu Phe Lys Ala Asp Ile His Gly Arg Thr
 20 25 30

Ala Leu Tyr Tyr Ala Ile Asp Asn Asn Asn Val Arg Leu Val Cys Glu
 35 40 45

Leu Leu Asn Ala Gly Val Leu Lys Asn Leu Leu Glu Asn Glu Phe Pro
 50 55 60

Leu His Gln Ala Ala Lys Leu Glu Asp Thr Lys Ile Val Lys Ile Leu
 65 70 75 80

Leu Phe Ser Gly Met Asp Asp Ser Gln Phe Asp Asp Arg Gly Asn Thr
 85 90 95

Pro Leu Tyr Tyr Ala Val Glu Ala Gly Asn Ile Gln Thr Ile Lys Leu
 100 105 110

Phe Ile Lys Lys Lys Trp Lys Leu Met Phe Tyr Gly Lys Thr Gly Trp
 115 120 125

Lys Thr Pro Phe Tyr Lys Ala Val Met Leu Asn Asp Val Ser Ile Thr
 130 135 140

Glu Tyr Phe Ile Ser Glu Leu Lys Ala Ser Phe Asp Leu Ala Ile Leu
 145 150 155 160

Tyr Ser Cys Ile His Asp Thr Ile Lys Asn Gly Asn Val Asp Met Leu
 165 170 175

```

Ile Leu Leu Leu Asp Tyr Met Met Ala Thr Asn Thr Asn Asn Ser Leu
      180                      185                      190

Leu Phe Ile Pro Asp Ile Lys Leu Ala Ile Glu Asn Lys Asp Leu Asp
      195                      200                      205

Leu Leu Arg Thr Leu Phe Lys Tyr Asp Ile Asn Ile Tyr Ser Val Asn
      210                      215                      220

Leu Glu Asn Val Leu Leu Asp Asp Pro Val Ile Ala Arg Met Ile Ile
      225                      230                      235                      240

Glu Lys His Val Asp Tyr Lys Thr Asp Asp Leu Ile Lys Glu Leu Asp
      245                      250                      255

Val Val Lys Asn Asn Lys Leu Asp Glu Ile Ile Ser Lys Asn Lys Glu
      260                      265                      270

Leu Arg Ile Met Tyr Val Asn Ser Ala Lys Ile Phe Arg Ser Gln Phe
      275                      280                      285

```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 173 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

```

Pro Thr Met Asn Leu Val Ala Lys Leu Gln Gly Asn Thr Ile Thr Val
1          5          10          15

Asp Asp Tyr Glu Tyr Asp Met Val Arg Leu Pro Tyr Lys Gly Ser Asn
      20          25          30

Val Ser Met Tyr Ile Ala Ile Gly Asp Asn Ile Thr His Phe Val Asp
      35          40          45

Ser Ile Thr Thr Thr Lys Leu Asp Tyr Trp Ser Ser Gln Leu Met Asp
      50          55          60

Lys Met Tyr Asp Leu Ser Leu Pro Arg Phe Ser Ile Glu Asn Lys Arg
      65          70          75          80

Asp Ile Lys Thr Ile Ala Glu Met Ile Ala Pro Ser Met Phe Asn Pro
      85          90          95

Asp Asn Ala Ser Phe Lys Arg Met Thr Lys Tyr Pro Leu Tyr Ile Tyr
      100         105         110

Lys Met Phe Gln Asn Ala Lys Ile Asp Val Asp Glu Gln Gly Thr Val
      115         120         125

```

Ala Glu Ala Ser Thr Ile Met Leu Ala Thr Gly Arg Ser Ala Pro Val
 130 135 140

Glu Leu Glu Phe Asn Lys Pro Phe Val Phe Ile Ile Arg His Asp Ile
 145 150 155 160

Thr Gly Phe Ile Leu Phe Ile Gly Lys Val Glu Ser Pro
 165 170

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 672 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:

- (A) ORGANISM: Raccoonpox virus ATCC# VR-838

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 95..547

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

TATAACAGCT CGTGGAGTTG CTATATTATT TTTATGTTTT ATTGATAATT GTAAAAAAA	60
CACACAATTA AATCGATAAG ATTGGGTAAT AACCATGGAG AGTAAATTAG ATTACGAGGA	120
CGCTGTTTTT TACTTTGTAT ACGACGACGA GTTATGCAGT CGTGACCTCA TTATCGATCT	180
AATTGATGAA TACGTCACGT GGAGAAATTA TATTATTGTA TTCGGTAAAG ATATTAACAA	240
ATGCGGAAGA CTATACAAGG AGTTGATGAA ATTTGACGAT GTTGCCATTA ATTACTATGG	300
GATGGATAAG ATCAATGAGA TTATTGATAC TATGAATGTC GGAGATAGAT ATATTAATCT	360
TAAAGAAGTC CATGATCAGG AAAGTCTGTT TGCCACCATC GGTATATGTG CAAAATCAC	420
TGAAAATTGG GGTATAAAA ATTTTTCAGA ATCTAGATTT CAATCATTAAG GAAATATTAA	480
AGATTTGATG ACTGATGACA ACATAAATAC GTTGATGATT TTTCTAGAAA AAAAATTAGC	540
AGTATAATAT AGTTTTTTAT AAAAAGCATT AAATAGACTC GTATTATATT TTTGTTAAAA	600
AATTCATTAA CCCATTATAT ATTTTAAAGT CTTATATGTC ACAAACATGA AAACGTGAAT	660
TCCTATAAGC TT	672

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 150 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

```

Met Glu Ser Lys Leu Asp Tyr Glu Asp Ala Val Phe Tyr Phe Val Tyr
 1             5             10             15

Asp Asp Glu Leu Cys Ser Arg Asp Leu Ile Ile Asp Leu Ile Asp Glu
 20             25             30

Tyr Val Thr Trp Arg Asn Tyr Ile Ile Val Phe Gly Lys Asp Ile Asn
 35             40             45

Lys Cys Gly Arg Leu Tyr Lys Glu Leu Met Lys Phe Asp Asp Val Ala
 50             55             60

Ile Asn Tyr Tyr Gly Met Asp Lys Ile Asn Glu Ile Ile Asp Thr Met
 65             70             75             80

Asn Val Gly Asp Arg Tyr Ile Asn Leu Lys Glu Val His Asp Gln Glu
 85             90             95

Ser Leu Phe Ala Thr Ile Gly Ile Cys Ala Lys Ile Thr Glu Asn Trp
100            105            110

Gly Tyr Lys Asn Phe Ser Glu Ser Arg Phe Gln Ser Leu Gly Asn Ile
115            120            125

Lys Asp Leu Met Thr Asp Asp Asn Ile Asn Thr Leu Met Ile Phe Leu
130            135            140

Glu Lys Lys Leu Ala Val
145            150

```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Raccoonpox virus ATCC# VR-838

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

CCGAAGCTTC CGTGCTCCAT CTATATAATA TATTAAAC

38

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Raccoonpox virus ATCC# VR-838

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

AATAAGCTTC CCGTTACTTT AGTAAAATCT TTTACAAA

38

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swine influenza virus

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

CCGAGGATCC GGCAATACTA TTAGTCTTGC TATGTACATT

40

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swine influenza virus

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

CTCTGGGATC CTAATTTTAA ATACATATTC TGCACTGTA

39

2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swine influenza virus

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

CCGAGGATCC GGCAATACTA TTAGTCTTGC TATGTACAT

39

2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swine influenza virus

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

CTCTGGATCC TAATTTAAAT ACATATTCTG CACTGTA

37

2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swine influenza virus

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

AATGAATTCA AATCAAAAAA TAATAACCAT TGGGTCAAT 39

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Swine influenza virus

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

GGAAGATCTA CTTGTCAATG GTGAATGGCA GATCAG 36

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Porcine respiratory reproductive syndrome virus

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

TTCGAATTCG GCTAATAGCT GTACATTCCT CCATATTT 38

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Porcine respiratory reproductive syndrome virus
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGGATCCTA TCGCCGTACG GCACTGAGGG

30

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Porcine respiratory reproductive syndrome virus
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCGAATTCGG CTGCGTCCCT TCTTTTCCTC ATGG

34

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Porcine respiratory reproductive syndrome virus
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTGGATCCTT CAAATTGCCA ACAGAATGGC AAAAAGAC

38

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Porcine respiratory reproductive syndrome virus

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

TTGAATTCGT TGGAGAAATG CTTGACCGCG GGC

33

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Porcine respiratory reproductive syndrome virus

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

GAAGGATCCT AAGGACGACC CCATTGTTCC GCTG

34

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 201 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Escherichia coli

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```

AGATCTTCCA TAATTAATTA ACCCTCGACT CTAGATTTTT TTTTTTTTTT TTTTGGCAT      60
ATAAATAGAT CTGTATCCTA AAATTGAATT GTAATTATCG ATAATAAATG AATTCGGATC      120
CATAATTAAT TAATTTTAT CCCGGCGCGC CGGGTCGAGG GTCGACCTGC AGGGCGGCCG      180
CGGCCCTCGA GGCCAAGCTT G                                          201

```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 189 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Escherichia coli

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```

AGATCTTCCA TAATTAATTA ACCCTCGACT CTAGATTTTT TTTTTTTTTT TTTTGGCAT      60
ATAAATAGAT CTGTATCCTA AAATTGAATT GTAATTATCG ATAATAAATG AATTCGGATC      120
CATAATTAAT TAATTTTAT CCCGGCGCGC CGGGTCGAGG GTCGACCTGC AGGGCGGCCG      180
CGGCCCTCG                                          189

```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 90 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Raccoonpox virus ATCC# VR-838

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

```

ATAGGTAAGA CGGCACAGTA CCTTTGTCGA CCATCTCCTC CACATTTATA TCATCCAATA      60
TTATTGAAAT GGTGGTAATA GACAAATGAT                                          90

```

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Porcine parvovirus

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

CTAAGGATCC GAGTGAAAAT GTGGAACAAC ACAACCC

37

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Porcine parvovirus

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

GTGGATCCTA GTATAATTTT CTTGGTATAA GTTGTGAA

38

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2375 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..738

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 799..2375

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: 1106

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: 1109

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

AAGCTTGTTA AACATGGCGA TTTATGGATA GCCAATTATA CATCTAAAGA CAGTCACCGT	60
AGGTATTTGT GTACTGTAAC GACAAAGAAT GGAGATTGTG TTCAGGGTAT AGTTAGATCT	120
CATATTAGGA AACCTTCATG TATTCCACAA ACCTATGAAC TAGGTACTCA CGATAAGTAT	180
GGTATAGACT TATATTGTGG AATTCTTTAC GCAAACCATT ATGATAATAT AACTTGGTAT	240
AAAAATAATC AAGAAATCAA TATCGAOGGT ATTAAGTATT CTCAATCGGA TAAGAATTTA	300
ATTATTCACA GTCCAGAGTT AGAAGATAGT GGAAGATACA ATTGTTATGT TCACTACAAT	360
GACGTTAAAA ACAAGGACGA TATCATCGTA TCAAGATGTA GAATGCTTAC AGTTATACCG	420
TCACAAGACC ACAGGTTTAA ACTAATACTA GATCCGAAAA TCAATGCGAC AATAGGAAAA	480
CCTGCCAACA TAACATGTAT GCGCGTTACT AGTTCGCTAT CGATTGATGA TTTACTAATA	540
GAATGGGAAG ATCCATCCGG ATGGTTTATA GGATTGATT TTGATGTATA TGCGGTTTTA	600
TATAGTGAAG GTGGTGGTAT CACCAGGTCG ACATTGTATT TTGAAAATGT TACTGAAGAA	660
TATATAGGTA ATACATATAC CTGTCGTGGA CACAACATAT ATTTTCAGAA AACCCTTACG	720
ACCACAGTAG TATTGGAGTA AACAACTAAC ATTTTATAT TACTGAAATT TAACGTATAA	780
TCCTCGTTTC TATATAAAAT GGATGAAGAT AGGATGCTAC TAACTAAGTA TATGTATCTC	840
ACTGATAGAG AACATATAAA TATAGACTCT GTTAAACGGT TATGTGAAAT ATCGGATCCT	900
AATGCGTGTT ATAGATCAGG ATGTACCGCT TTACACGAGT ACTTTTATAA TTGTAGATCA	960
ATCAAAGGAA AATACGATTA TAGGTATAAT GGTATTATA AATATTATTA TTCAGGAGAT	1020
TATGAAAAC ATAACAGTAA TTATGATCAT GCTATAGATA TGACAGACGA TGACGACACG	1080
GAAGATGATG AATCATCTGA AACAAATNT GAATTTTATG ATGAAACACA AGATCAAAAT	1140
AATCAGCTAA TATGTTCTAA TATTAACTC ACAAATAGAG TAGATGATTT CGTAGATGAA	1200
TTCTATGGTT ACGATCAGAA TATCGACATA AATGATTATG TAGATAAATC TATAAAACAT	1260

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GTAGTATATG GAAGCGATAA TAGGCGAATG CGATGGTGTG ATGTATGGAG ATGTCACCAT 1320
AGCGGATCAT ACAGATTCGG TAAGGAATGT ATAGACAATA TATATGAAGA TAATAATACA 1380
AAAATAGAAT TCGATAATTT CGATTCGTTG TCAAACCTTA CTGATGCGGA ATATGTAATT 1440
CGGGTAACTA GAGATGCGTC TACACAAATA TGGGAAAAGA AATCAGTGTT AGATAGATAC 1500
ATGGAGTCAT ATAGCCGTAA CCGATATAGT AAACATTCTG TCTTTAAGGG ATTTTCTGAT 1560
TACGTTAGAA AAAATGATTT AGATATGAAT ATTGTTAAAG AACTACTTTC GAACGGCGCA 1620
TCGCTGACAA TCAATGATGG AAGTCGTTGG GACCCCATCT TAGTGTACTT CAGAAGAACT 1680
ATAATGAATT TAGACATGAT CGATATTATT AACAAATCATA CAACTATAGA TGAACGAAAG 1740
TATATAGTAC ACATCTATCT AAACAATTAC AGAAATTTCTG ATTACCCATT CTTTAGGAAG 1800
TTAGTCATAA CCAATAAACA TTGTCTTAAG AATTATTATA CTAATCATGA TGATATATAC 1860
GGCACACCAC TTCATATGTT ATCATCTAAT AAAAAATTAA TAACGCCTAA TTACATTAAA 1920
TTATTGGTAT ATAACGGAAA TGATATAAAC GCGCGAGGGA ATGAGTCATA TATGAGAACT 1980
CCATTACATC ATTATTTAAG TAAATGGTT TATCACGATA GAGAATATGA TGTTGGATAT 2040
TATAATGAAA AGATTATAGA TGCCTTTATA GAATTAGGAG CCGATCTAAC GATTGCGGAT 2100
AATAATGAGA TGATACCAGT AATTCATGCT ATTCATAGAA ATCCGCATG TGATGGTTAC 2160
ATCAATACCA ATAATATAAA GATAATAAGT AACTACTTA ATCTCAGTAG ACACGCACCG 2220
CATAATCTAT TTAGGGATCG CGTAATGCAT GATTATATAA TTAATTCATA TACTAATCTT 2280
GAATGTTTGG ATATTATTAG ATCACTGGAT GGATTTGATA TTAATTGTTA CTTTAACGGA 2340
CATACACCAC TTCATTGTGC CATAATTAAA AGCTT 2375

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(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 246 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

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Lys Leu Val Lys His Gly Asp Leu Trp Ile Ala Asn Tyr Thr Ser Lys
1           5           10           15
Asp Ser His Arg Arg Tyr Leu Cys Thr Val Thr Thr Lys Asn Gly Asp
20           25           30

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Cys Val Gln Gly Ile Val Arg Ser His Ile Arg Lys Pro Ser Cys Ile
      35                      40                      45

Pro Gln Thr Tyr Glu Leu Gly Thr His Asp Lys Tyr Gly Ile Asp Leu
      50                      55                      60

Tyr Cys Gly Ile Leu Tyr Ala Asn His Tyr Asp Asn Ile Thr Trp Tyr
      65                      70                      75                      80

Lys Asn Asn Gln Glu Ile Asn Ile Asp Gly Ile Lys Tyr Ser Gln Ser
      85                      90                      95

Asp Lys Asn Leu Ile Ile His Ser Pro Glu Leu Glu Asp Ser Gly Arg
      100                     105                     110

Tyr Asn Cys Tyr Val His Tyr Asn Asp Val Lys Asn Lys Asp Asp Ile
      115                     120                     125

Ile Val Ser Arg Cys Arg Met Leu Thr Val Ile Pro Ser Gln Asp His
      130                     135                     140

Arg Phe Lys Leu Ile Leu Asp Pro Lys Ile Asn Ala Thr Ile Gly Lys
      145                     150                     155                     160

Pro Ala Asn Ile Thr Cys Met Ala Val Thr Ser Ser Leu Ser Ile Asp
      165                     170                     175

Asp Leu Leu Ile Glu Trp Glu Asp Pro Ser Gly Trp Phe Ile Gly Phe
      180                     185                     190

Asp Phe Asp Val Tyr Ala Val Leu Tyr Ser Glu Gly Gly Gly Ile Thr
      195                     200                     205

Arg Ser Thr Leu Tyr Phe Glu Asn Val Thr Glu Glu Tyr Ile Gly Asn
      210                     215                     220

Thr Tyr Thr Cys Arg Gly His Asn Tyr Tyr Phe Gln Lys Thr Leu Thr
      225                     230                     235                     240

Thr Thr Val Val Leu Glu
      245

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(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 525 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

Met Asp Glu Asp Arg Met Leu Leu Thr Lys Tyr Met Tyr Leu Thr Asp

1	5	10	15
Arg Glu His	Ile Asn Ile Asp Ser Val Lys Arg Leu Cys Glu Ile Ser		
	20	25	30
Asp Pro Asn Ala Cys Tyr Arg Ser Gly Cys Thr Ala Leu His Glu Tyr			
	35	40	45
Phe Tyr Asn Cys Arg Ser Ile Lys Gly Lys Tyr Asp Tyr Arg Tyr Asn			
	50	55	60
Gly Tyr Tyr Lys Tyr Tyr Tyr Ser Gly Asp Tyr Glu Asn Tyr Asn Ser			
	65	70	75
Asn Tyr Asp His Ala Ile Asp Met Thr Asp Asp Asp Asp Thr Glu Asp			
	85	90	95
Asp Glu Ser Ser Glu Thr Xaa Xaa Glu Phe Tyr Asp Glu Thr Gln Asp			
	100	105	110
Gln Asn Asn Gln Leu Ile Cys Ser Asn Ile Lys Leu Thr Asn Arg Val			
	115	120	125
Asp Asp Phe Val Asp Glu Phe Tyr Gly Tyr Asp Gln Asn Ile Asp Ile			
	130	135	140
Asn Asp Tyr Val Asp Lys Ser Ile Lys His Val Val Tyr Gly Ser Asp			
	145	150	155
Asn Arg Arg Met Arg Trp Cys Asp Val Trp Arg Cys His His Ser Gly			
	165	170	175
Ser Tyr Arg Phe Gly Lys Glu Cys Ile Asp Asn Ile Tyr Glu Asp Asn			
	180	185	190
Asn Thr Lys Ile Glu Phe Asp Asn Phe Asp Ser Leu Ser Asn Leu Thr			
	195	200	205
Asp Ala Glu Tyr Val Ile Arg Val Thr Arg Asp Ala Ser Thr Gln Ile			
	210	215	220
Trp Glu Lys Lys Ser Val Leu Asp Arg Tyr Met Glu Ser Tyr Ser Arg			
	225	230	235
Asn Arg Tyr Ser Lys His Ser Val Phe Lys Gly Phe Ser Asp Tyr Val			
	245	250	255
Arg Lys Asn Asp Leu Asp Met Asn Ile Val Lys Glu Leu Leu Ser Asn			
	260	265	270
Gly Ala Ser Leu Thr Ile Asn Asp Gly Ser Arg Trp Asp Pro Ile Leu			
	275	280	285
Val Tyr Phe Arg Arg Thr Ile Met Asn Leu Asp Met Ile Asp Ile Ile			
	290	295	300
Asn Asn His Thr Thr Ile Asp Glu Arg Lys Tyr Ile Val His Ile Tyr			
	305	310	315
			320

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Leu Asn Asn Tyr Arg Asn Phe Asp Tyr Pro Phe Phe Arg Lys Leu Val
      325                      330                      335
Ile Thr Asn Lys His Cys Leu Lys Asn Tyr Tyr Thr Asn His Asp Asp
      340                      345                      350
Ile Tyr Gly Thr Pro Leu His Met Leu Ser Ser Asn Lys Lys Leu Ile
      355                      360                      365
Thr Pro Asn Tyr Ile Lys Leu Leu Val Tyr Asn Gly Asn Asp Ile Asn
      370                      375                      380
Ala Arg Gly Asn Glu Ser Tyr Met Arg Thr Pro Leu His His Tyr Leu
      385                      390                      395                      400
Ser Lys Met Val Tyr His Asp Arg Glu Tyr Asp Val Gly Tyr Tyr Asn
      405                      410                      415
Glu Lys Ile Ile Asp Ala Phe Ile Glu Leu Gly Ala Asp Leu Thr Ile
      420                      425                      430
Ala Asp Asn Asn Glu Met Ile Pro Val Ile His Ala Ile His Arg Asn
      435                      440                      445
Ser Ala Cys Asp Gly Tyr Ile Asn Thr Asn Asn Ile Lys Ile Ile Ser
      450                      455                      460
Lys Leu Leu Asn Leu Ser Arg His Ala Pro His Asn Leu Phe Arg Asp
      465                      470                      475                      480
Arg Val Met His Asp Tyr Ile Ile Asn Ser Tyr Thr Asn Leu Glu Cys
      485                      490                      495
Leu Asp Ile Ile Arg Ser Leu Asp Gly Phe Asp Ile Asn Cys Tyr Phe
      500                      505                      510
Asn Gly His Thr Pro Leu His Cys Ala Ile Ile Lys Ser
      515                      520                      525

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(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3295 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Porcine reproductive respiratory virus
(Eichelberger strain)
- (ix) FEATURE:
 - (A) NAME/KEY: CDS

(B) LOCATION: 1..738

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 799..2375

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..738

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 799..2375

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..738

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 799..2375

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

GAATTCCCGG	GCCCTGTCAT	TGAACCAACT	TTGGGCCTGA	ACTGAAATGA	AATGGGGGCT	60
ATGCAAAGCC	TTTTTCGACAA	AATTGGCCAA	CTTTTTGTGG	ATGCTTTTAC	GGAATTTTTG	120
GTGTCCATTG	TTGATATCAT	CATATTTTGT	GCCATTTTGT	TTGGCTTCAC	CATCGCAGGT	180
TGGCTGGTGG	TCTTCTGTCAT	CCGATTGGTT	TGCTCCGCGG	TACTCCGTGC	GCGCCCTACC	240
ATTCACCTTG	AGCAATTACA	GAAGATCCTA	TGAAGCCTTT	CTTTCTCAGT	GCCAGGTGGA	300
CATTCCCACC	TGGGGAATCA	AACATCCCTT	GGGGATGTTT	TGGCACCATA	AGGTGTCAAC	360
CCTGATTGAT	GAATGGTGT	CGCGTCGAAT	GTACCGCATC	ATGGAAAAAG	CAGGACAGGC	420
TGCCTGGAAA	CAGGTGGTGA	GGGAGGTTAC	GCTGTCTCGC	ATTAGTGGTT	TGGACGTGGT	480
GGCTCATTTT	CAGCATCTTG	CCGCCATTGA	AGCCGAGACC	TGTAAATATT	TGGCCTCTCG	540
GCTGCCCATG	CTACACAACC	TGCGCATGAC	AGGGTCAAAT	GTAACCATAG	TGTATAATAG	600
CACTTCAAAT	CAGGTGTTTG	CTATTTTTC	AACCCCTGGG	TCCCGGCCAA	AGCTTCATGA	660
TTTTTCAGCA	TGGCTAATAG	CTGTGCACTC	CTCCATATTT	TCCCTCCGTAG	CGGCTTCTTG	720
TACTCTTTTT	GTTGTGCTGT	GGTTGCGGAT	CCCAATGCTA	CGTTCTGTTT	TTGGTTTCCA	780
CTGGTTAGGG	GCAATTTTTC	TTTCGAACTC	ACGGTGAATT	ACACAGTGTG	CCCACCTTGC	840
CTCCTCCGGC	AAGCAGCCGC	TGAGATCTAC	GAACCCGGCA	GGTCTCTTTG	GTGCAGGATA	900
GGGCATGACC	GATGTGGTGA	GGACGATCAT	GACGAAC TAG	GGTTCATGGT	TCCGCTTGGC	960
CTCTCCAGCG	AAGGCCACTT	GACCAGTGTT	TACGCTTGGT	TGGCGTTCCT	GTCCTTCAGC	1020
TACACGGCCC	AGTTCCATCC	CGAGATATTT	GGGATAGGGA	ATGTGAGTAA	AGTTTATGTT	1080
GACATCAAGC	ACCAATTCAT	CTGCGCCGAA	CACGACGGGC	GGAACGCCAC	CCTGCTTCGC	1140
CATGACAATA	TTTCAGCCGT	GTTTCAGACC	TACTACCAAC	ATCAGGTCGA	TGGCGGCAAT	1200
TGGTTTTCACC	TGGAATGGCT	GCGTCCCTTC	TTTTCCCTCT	GGTTGGTTTT	AAATGTTTTCG	1260
TGGTTTCTCA	GGCGTTCGCC	TGCAAGCCAT	GTTTCAGTTC	GAGTCTTTCA	GACATCAAAA	1320
CCAACACTAC	CGCAGCATCA	AACTTTGTTG	TCCTCCAGGA	CGTCAGCTGC	CTTAGGCATG	1380
GCGACCCGTC	CTCTCCGGCG	ATTCGCAAAA	GCCCTCAGTG	CCGTACGGCG	ATAGGAACAC	1440
CCGTGTATAT	CACCACCACA	GCCAATGTGA	CAGATGAGAA	TTATTTACAT	TCTTCTGATC	1500
TCCTCATGCT	TTCTTCTTGC	CTTTTCTATG	CTTCTGAAAT	GAGTGAAAAG	GGGTTCAAGG	1560
TGGTATTTGG	CAATGTGTCA	GGCATCGTGG	CTGTGTGTGT	CAACTTTACC	AGCTACGTCC	1620
AACATGTCAA	GGAGTTCACC	CAACGCTCCT	TGGTGGTCTGA	TCATGTGCGG	CTGCTTCATT	1680
TCATGACACC	TGAGACCATG	AGGTGGGCAA	CCGTTTTAGC	CTGTCTTTTT	GCCATCCTGC	1740
TGGCAATTTG	AATGTTCAAG	TATGTTGGGG	AAATGCTTGA	CCGCGGGCTG	TTGCTCGCGA	1800
TTGCTTTCTT	TGTGGTTTAT	CGTGCCGTTT	TGGTTTGCTG	TGCTCGTCAA	CGCCAACAGC	1860
AGCAGCAGCT	CCCATTTTCA	GTCGATTTAT	AACTTGACGC	TATGTGAGCT	GAATGGCACA	1920
AATTGGCTGG	CTGAAAATTT	TGATTGGGCA	GTGGAGACTT	TTGTCATCTT	TCCCGTGTTG	1980

ACTCACATTG	TTTCCTATGG	TGCACTTACC	ACCAGCCATT	TCCTTGACAC	AGTTGGTCTG	2040
GTTGCTGTGT	CCACCGCCGG	TTTTTTTCAC	GGGCGGTATG	TCTTGAGTAG	CATCTACGCG	2100
GTCTGTGCCC	TGGGTGCGTT	GATTTGCTTC	GTCATCAGAT	TTGCGAAGAA	CTGCATGTCC	2160
TGGCGCTACT	CATGTACCAG	ATATACCAAC	TTCTTCTAG	ATACTAAGGG	CAGACTCTAT	2220
CGTTGGCGGT	CGCCTGTTAT	CATAGAGAAA	GGGGGTAAGG	TTGAGGTCGG	AGGCCACCTG	2280
ATCGACCTCA	AAAGAGTTGT	GCTTGATGGT	TCCGTGGCGA	CTCCTTTAAC	CAGAGTTTCA	2340
GCTGAACAAT	GGGGTCGTCC	CTAGACGACT	TTTGCAATGA	TAGCACGGCT	CCGCAAAAAG	2400
TGCTTCTGGC	GTTTTCCATC	ACCTACACGC	CAGTGATGAT	ATATGCTCTA	AAGGTAAGTC	2460
GCGGCCGACT	GCTAGGGCTT	CTGCACCTTT	TGATCTTTCT	GAATTGTGCT	TTTACCTTCG	2520
GGTACATGAC	ATTTCGTGCAC	TTTCAGAGCA	CAAATAAGGT	CGCGCTCACT	ATGGGAGCAG	2580
TAGTTGCACT	TCTTTGGGGG	GTGTACTCAG	CCATAGAAAC	CTGGAAATTC	ATCACCTCCA	2640
GATGCCGTTT	GTGCTTGCTA	GGCCGCAAGT	ACATCCTGGC	CCCTGCCCAC	CACGTCGAAA	2700
GTGCCGCGGG	CTTTCATCCG	ATTGCGGCAA	ATGATAACCA	CGCATTTGTC	GTCCGGCGTC	2760
CCGGCTCCAC	TACGGTTAAC	GGCACATTGG	TGCCCCGGGT	GAAAAGCCTC	GTGTTGGGTG	2820
GCAGAAAAGC	TGTTAAACAG	GGAGTGGTAA	ACCTTGTCAA	ATATGCCAAA	TAACAACGGC	2880
AAGCAGCAAA	AGAAAAAGAA	GGGGAATGGC	CAGCCAGTCA	ATCAGCTGTG	CCAAATGCTG	2940
GGTAAGATCA	TCGCCCAGCA	AAACCAGTCC	AGAGGCAAGG	GACCGGGGAA	GAAAAATAAG	3000
AAGAAAAACC	CGGAGAAGCC	CCATTTTCCT	CTAGCGACCG	AAGATGACGT	CAGGCATCAC	3060
TTCACCCCTA	GTGAGCGGCA	ATTGTGTCTG	TCGTCCGATC	AGACTGCCTT	TAACCAGGGC	3120
GCTGGAACTT	GTACCCTGTC	AGATTCAGGG	AGGATAAGTT	ACACTGTGGA	GTTTAGTTTG	3180
CCGACGCATC	ATACTGTGCG	TCTGATTCGC	GCTACAGCAT	CACCCACAGC	GTGATGGGCT	3240
GACATTCTTG	AAGCACCTCA	GTGTTTGAAT	TGGAAGAATG	CGTGGTGAAG	GATCC	3295

2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 256 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

Met	Lys	Trp	Gly	Leu	Cys	Lys	Ala	Phe	Ser	Thr	Lys	Leu	Ala	Asn	Phe	1	5	10	15
Leu	Trp	Met	Leu	Ser	Arg	Asn	Phe	Trp	Cys	Pro	Leu	Leu	Ile	Ser	Ser	20	25	30	
Tyr	Phe	Trp	Pro	Phe	Cys	Leu	Ala	Ser	Pro	Ser	Gln	Val	Gly	Trp	Trp	35	40	45	
Ser	Ser	Ala	Ser	Asp	Trp	Phe	Ala	Pro	Arg	Tyr	Ser	Val	Arg	Ala	Leu	50	55	60	
Pro	Phe	Thr	Leu	Ser	Asn	Tyr	Arg	Arg	Ser	Tyr	Glu	Ala	Phe	Leu	Ser	65	70	75	80
Gln	Cys	Gln	Val	Asp	Ile	Pro	Thr	Trp	Gly	Ile	Lys	His	Pro	Leu	Gly	85	90	95	
Met	Phe	Trp	His	His	Lys	Val	Ser	Thr	Leu	Ile	Asp	Glu	Met	Val	Ser	100	105	110	

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Arg Arg Met Tyr Arg Ile Met Glu Lys Ala Gly Gln Ala Ala Trp Lys
    115                      120                      125

Gln Val Val Arg Glu Val Thr Leu Ser Arg Ile Ser Gly Leu Asp Val
    130                      135                      140

Val Ala His Phe Gln His Leu Ala Ala Ile Glu Ala Glu Thr Cys Lys
    145                      150                      155                      160

Tyr Leu Ala Ser Arg Leu Pro Met Leu His Asn Leu Arg Met Thr Gly
                      165                      170                      175

Ser Asn Val Thr Ile Val Tyr Asn Ser Thr Ser Asn Gln Val Phe Ala
                      180                      185                      190

Ile Phe Pro Thr Pro Gly Ser Arg Pro Lys Leu His Asp Phe Gln Gln
    195                      200                      205

Trp Leu Ile Ala Val His Ser Ser Ile Phe Ser Ser Val Ala Ala Ser
    210                      215                      220

Cys Thr Leu Phe Val Val Leu Trp Leu Arg Ile Pro Met Leu Arg Ser
    225                      230                      235                      240

Val Phe Gly Phe His Trp Leu Gly Ala Ile Phe Leu Ser Asn Ser Arg
                      245                      250                      255

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(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 254 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```

Met Ala Asn Ser Cys Ala Leu Leu His Ile Phe Leu Arg Ser Gly Phe
1          5          10          15

Leu Tyr Ser Phe Cys Cys Ala Val Val Ala Asp Pro Asn Ala Thr Phe
    20          25          30

Cys Phe Trp Phe Pro Leu Val Arg Gly Asn Phe Ser Phe Glu Leu Thr
    35          40          45

Val Asn Tyr Thr Val Cys Pro Pro Cys Leu Thr Arg Gln Ala Ala Ala
    50          55          60

Glu Ile Tyr Glu Pro Gly Arg Ser Leu Trp Cys Arg Ile Gly His Asp
    65          70          75          80

Arg Cys Gly Glu Asp Asp His Asp Glu Leu Gly Phe Met Val Pro Pro

```

85										90					95		
Gly	Leu	Ser	Ser	Glu	Gly	His	Leu	Thr	Ser	Val	Tyr	Ala	Trp	Leu	Ala		
			100					105					110				
Phe	Leu	Ser	Phe	Ser	Tyr	Thr	Ala	Gln	Phe	His	Pro	Glu	Ile	Phe	Gly		
		115					120					125					
Ile	Gly	Asn	Val	Ser	Lys	Val	Tyr	Val	Asp	Ile	Lys	His	Gln	Phe	Ile		
	130					135					140						
Cys	Ala	Glu	His	Asp	Gly	Arg	Asn	Ala	Thr	Leu	Leu	Arg	His	Asp	Asn		
145					150					155					160		
Ile	Ser	Ala	Val	Phe	Gln	Thr	Tyr	Tyr	Gln	His	Gln	Val	Asp	Gly	Gly		
				165					170					175			
Asn	Trp	Phe	His	Leu	Glu	Trp	Leu	Arg	Pro	Phe	Phe	Ser	Ser	Trp	Leu		
			180					185					190				
Val	Leu	Asn	Val	Ser	Trp	Phe	Leu	Arg	Arg	Ser	Pro	Ala	Ser	His	Val		
		195					200					205					
Ser	Val	Arg	Val	Phe	Gln	Thr	Ser	Lys	Pro	Thr	Leu	Pro	Gln	His	Gln		
	210					215					220						
Thr	Leu	Leu	Ser	Ser	Arg	Thr	Ser	Ala	Ala	Leu	Gly	Met	Ala	Thr	Arg		
225					230					235					240		
Pro	Leu	Arg	Arg	Phe	Ala	Lys	Ala	Leu	Ser	Ala	Val	Arg	Arg				
				245					250								

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 178 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met	Ala	Ala	Ser	Leu	Leu	Phe	Leu	Leu	Val	Gly	Phe	Lys	Cys	Phe	Val
1				5					10					15	
Val	Ser	Gln	Ala	Phe	Ala	Cys	Lys	Pro	Cys	Phe	Ser	Ser	Ser	Leu	Ser
			20					25					30		
Asp	Ile	Lys	Thr	Asn	Thr	Thr	Ala	Ala	Ser	Asn	Phe	Val	Val	Leu	Gln
		35					40					45			
Asp	Val	Ser	Cys	Leu	Arg	His	Gly	Asp	Pro	Ser	Ser	Pro	Ala	Ile	Arg
	50					55					60				

Lys Ser Pro Gln Cys Arg Thr Ala Ile Gly Thr Pro Val Tyr Ile Thr
 65 70 75 80
 Thr Thr Ala Asn Val Thr Asp Glu Asn Tyr Leu His Ser Ser Asp Leu
 85 90 95
 Leu Met Leu Ser Ser Cys Leu Phe Tyr Ala Ser Glu Met Ser Glu Lys
 100 105 110
 Gly Phe Lys Val Val Phe Gly Asn Val Ser Gly Ile Val Ala Val Cys
 115 120 125
 Val Asn Phe Thr Ser Tyr Val Gln His Val Lys Glu Phe Thr Gln Arg
 130 135 140
 Ser Leu Val Val Asp His Val Arg Leu Leu His Phe Met Thr Pro Glu
 145 150 155 160
 Thr Met Arg Trp Ala Thr Val Leu Ala Cys Leu Phe Ala Ile Leu Leu
 165 170 175
 Ala Ile

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 200 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Leu Gly Lys Cys Leu Thr Ala Gly Cys Cys Ser Arg Leu Leu Ser
 1 5 10 15
 Leu Trp Phe Ile Val Pro Phe Trp Phe Ala Val Leu Val Asn Ala Asn
 20 25 30
 Ser Ser Ser Ser Ser His Phe Gln Ser Ile Tyr Asn Leu Thr Leu Cys
 35 40 45
 Glu Leu Asn Gly Thr Asn Trp Leu Ala Glu Lys Phe Asp Trp Ala Val
 50 55 60
 Glu Thr Phe Val Ile Phe Pro Val Leu Thr His Ile Val Ser Tyr Gly
 65 70 75 80
 Ala Leu Thr Thr Ser His Phe Leu Asp Thr Val Gly Leu Val Ala Val
 85 90 95
 Ser Thr Ala Gly Phe Phe His Gly Arg Tyr Val Leu Ser Ser Ile Tyr
 100 105 110

Ala Val Cys Ala Leu Ala Ala Leu Ile Cys Phe Val Ile Arg Phe Ala
 115 120 125

Lys Asn Cys Met Ser Trp Arg Tyr Ser Cys Thr Arg Tyr Thr Asn Phe
 130 135 140

Leu Leu Asp Thr Lys Gly Arg Leu Tyr Arg Trp Arg Ser Pro Val Ile
 145 150 155 160

Ile Glu Lys Gly Gly Lys Val Glu Val Gly Gly His Leu Ile Asp Leu
 165 170 175

Lys Arg Val Val Leu Asp Gly Ser Val Ala Thr Pro Leu Thr Arg Val
 180 185 190

Ser Ala Glu Gln Trp Gly Arg Pro
 195 200

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 174 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Gly Ser Ser Leu Asp Asp Phe Cys Asn Asp Ser Thr Ala Pro Gln
 1 5 10 15

Lys Val Leu Leu Ala Phe Ser Ile Thr Tyr Thr Pro Val Met Ile Tyr
 20 25 30

Ala Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Leu Leu
 35 40 45

Ile Phe Leu Asn Cys Ala Phe Thr Phe Gly Tyr Met Thr Phe Val His
 50 55 60

Phe Gln Ser Thr Asn Lys Val Ala Leu Thr Met Gly Ala Val Val Ala
 65 70 75 80

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

Ser Arg Cys Arg Leu Cys Leu Leu Gly Arg Lys Tyr Ile Leu Ala Pro
 100 105 110

Ala His His Val Glu Ser Ala Ala Gly Phe His Pro Ile Ala Ala Asn
 115 120 125

Asp Asn His Ala Phe Val Val Arg Arg Pro Gly Ser Thr Thr Val Asn
 130 135 140

Gly Thr Leu Val Pro Gly Leu Lys Ser Leu Val Leu Gly Gly Arg Lys
 145 150 155 160

Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Lys Ala
 165 170

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Pro Asn Asn Asn Gly Lys Gln Gln Lys Lys Lys Lys Gly Asn Gly
 1 5 10 15

Gln Pro Val Asn Gln Leu Cys Gln Met Leu Gly Lys Ile Ile Ala Gln
 20 25 30

Gln Asn Gln Ser Arg Gly Lys Gly Pro Gly Lys Lys Asn Lys Lys Lys
 35 40 45

Asn Pro Glu Lys Pro His Phe Pro Leu Ala Thr Glu Asp Asp Val Arg
 50 55 60

His His Phe Thr Pro Ser Glu Arg Gln Leu Cys Leu Ser Ser Ile Gln
 65 70 75 80

Thr Ala Phe Asn Gln Gly Ala Gly Thr Cys Thr Leu Ser Asp Ser Gly
 85 90 95

Arg Ile Ser Tyr Thr Val Glu Phe Ser Leu Pro Thr His His Thr Val
 100 105 110

Arg Leu Ile Arg Ala Thr Ala Ser Pro Thr Ala
 115 120

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTGAGAATTC ATGGGGGCTA TGCAAAGCCT TTTCG

35

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTGGAATTCA CCGTGAGTTC GAAAGAAAAA TTGC

34

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CAATGAATTC TAGCTGTGCA CTCCTCCATA TTTTCCTC

38

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

38

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CGGGAATTCC TATCGCCGTA CGGCACTGAG G

31

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGGAATTCTG CGTCCCTTCT TTCCTCTTG GTTG

34

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CTTGATCCT CAAATTGCCA GCAGGATGGC AAAAAG

36

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

39

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CAAGGAATTC GGGGAAATGC TTGACCGCGG GCTG

34

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

AAAAGGATCC TAGGGACGAC CCCATTGTTC AGC

33

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CAATGAATTC GTCCCTAGAC GACTTTTGCA ATGATAG

37

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CTTGGGATCC TTATTTGGCA TATTTGACAA GGTTTACCAC

40

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

TATGAATTCT AACAAACGGCA AGCAGCAAAA GAAAAAG

37

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TGTGGATCCA TCACGCTGTG GGTGATGCTG TAG

33

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```

CTGGTGCCGG AAACCAGGCA AAGCGCCATT CGCCATTCAG GCTGCGCAAC TGTGCGGAAG      60
GGCGATCGGT GCGGGCCTCT TCGCTATTAC GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA      120
GGCGATTAAG TTGGGTAACG CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGGAT      180
CTGTCATTTA TATAAATTAA TTCTAAATAA TATAGGATCC CGATCTTGTC GTATATGTTG      240
TGTTATTGAA TGC GTGTTTT CTACAAACTC TTTTATGCGT AGAAACTTTT AACGTTGGTG      300
TATCAAATGT CTAGAACTAG TGGATCCCCC GGG                                     333

```

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

```

TTGGGCGCGC CATTTATCTA TTGATCACAT CGATCAG                                37

```

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CCGGCGCGCC AGACTGCAAC AGATCTACTA GACAAGAGTC

40

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Synthetic

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CTGGTGCCGG AAACCAGGCA AAGCGCCATT CGCCATTCAG GCTGCGCAAC TGTGGGAAG	60
GGCGATCGGT GCGGGCCTCT TCGCTATTAC GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA	120
GGCGATTAAG TTGGGTAACG CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACGGGAT	180
CTGTCATTTA TATAAATTAA TTCTAAATAA TATAGGATCC CGATCTTGTC GTATATGTTG	240
TGTTATTGAA TGC GTGTTTT CTACAACTC TTTTATGCGT AGAAACTTTT AACGTTGGTG	300
TATCAAATGT CTAGAACTAG TGGATCCCCC GGG	333

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 174 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```

Met Gly Ser Ser Leu Asp Asp Phe Cys Asn Asp Ser Thr Ala Pro Gln
 1             5             10             15

Lys Val Leu Leu Ala Phe Ser Ile Thr Tyr Thr Pro Val Met Ile Tyr
      20             25             30

Ala Leu Lys Val Ser Arg Gly Arg Leu Leu Gly Leu Leu His Leu Leu
 35             40             45

Ile Phe Leu Asn Cys Ala Phe Thr Phe Gly Tyr Met Thr Phe Val His
 50             55             60

Phe Gln Ser Thr Asn Lys Val Ala Leu Thr Met Gly Ala Val Val Ala
 65             70             75             80

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

Ser Arg Cys Arg Leu Cys Leu Leu Gly Arg Lys Tyr Ile Leu Ala Pro
      100            105            110

Ala His His Val Glu Ser Ala Ala Gly Phe His Pro Ile Ala Ala Asn
      115            120            125

Asp Asn His Ala Phe Val Val Arg Arg Pro Gly Ser Thr Thr Val Asn
      130            135            140

Gly Thr Leu Val Pro Gly Leu Lys Ser Leu Val Leu Gly Gly Arg Lys
      145            150            155            160

Ala Val Lys Gln Gly Val Val Asn Leu Val Lys Tyr Ala Lys
      165            170

```

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 688 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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

Lys Leu Met Ile Glu Tyr Asp Leu Leu Ser Arg Ser Asp Leu Glu Trp
 1 5 10 15
 Leu Ile Arg Glu Asn Ile Val Arg Ala Thr His Leu Leu Lys Ile Asn
 20 25 30
 Ala Tyr Met Ile Asn Phe Lys Ile Asp Leu Thr Val Asp Glu Ile Arg
 35 40 45
 Asp Val Val Lys Glu Ile Pro Val Gly Ser Val Leu His Leu Tyr Asn
 50 55 60
 Ile Leu Asn Asn Ile Asp Leu Glu Ile Val Ile Ser Ile Ser Asp Glu
 65 70 75 80
 Tyr Asn Val Pro Pro Ile His Asp Val Leu His Asn Leu Thr Asp Glu
 85 90 95
 Gln Ile Cys Ile Asn Leu Val Lys Lys Tyr Pro Leu Asp Asn Val Ile
 100 105 110
 Asn Phe Ile Arg Gln Asp Ile Lys Tyr Ser Pro Ser Phe Ile Ser Ala
 115 120 125
 Val Lys Gln Phe Val Asn Glu Asn Leu Pro Thr Met Tyr Asp Gly Leu
 130 135 140
 Asn Asp Tyr Leu Tyr Ser Val Ile Val Glu Glu Glu Leu Ile Asp Glu
 145 150 155 160
 Tyr Lys Ile Lys Ser Val Ala Met Phe Asn Leu Gly Tyr Glu Thr Glu
 165 170 175
 Ile Asp Met Leu Thr Leu Asp Glu Arg Lys Phe Val Glu Thr Asn Ile
 180 185 190
 Ala Tyr Tyr Asp Phe Arg Tyr Arg Arg Phe Ala Asp Glu Phe Arg Asn
 195 200 205
 Phe Ile Met Lys Thr Glu Arg Arg Leu Ile Thr Met Pro Ser Gly Asp
 210 215 220
 Arg Val Arg Arg Ser Arg Arg Pro Lys Ser Leu Arg Ser Thr Ile Ile
 225 230 235 240
 Lys Lys Asp Thr Asp Ser Leu Glu Asp Val Leu Glu His Ile Asp Asn
 245 250 255
 Ala Arg Arg Asn Ser Lys Leu Ser Ile Gly Asp Val Glu Arg Ile Ile
 260 265 270
 Ser Ser Phe Arg Leu Asn Pro Cys Ile Val Arg Arg Val Met Leu Ser
 275 280 285
 Ser Ile Asp Ile Lys Thr Lys Ile Met Val Ile Lys Ile Val Lys Asp
 290 295 300

Trp Lys Ser Cys Ser Leu Thr Leu Ser Ala Ile Lys Gly Ile Met Val
 305 310 315 320
 Thr Asp Thr Ile Asn Thr Val Phe Ser Lys Ile Leu His Tyr His Arg
 325 330 335
 Lys Ile Phe Lys Tyr Ile Thr Ser Val Asp Asp Lys Asp Ile Thr Ser
 340 345 350
 Cys Cys Cys Ser Arg Cys Ile Asn Leu Phe Tyr Arg Glu Leu Lys Ser
 355 360 365
 Ile Val Cys Asn Leu Asp Thr Asp Asp Gly Leu Leu Asp Arg Leu Tyr
 370 375 380
 Asp Leu Thr Arg Tyr Ala Ile His Gly Lys Ile Asn Gln Lys Leu Ile
 385 390 395 400
 Gly Gln Arg Cys Trp Gly Pro Leu Thr Glu Met Met Phe Asn Glu Val
 405 410 415
 Lys Lys Lys Lys Leu Asn Asp Leu Met Glu His Ile Arg Ile Ser Asp
 420 425 430
 Met Leu Val Tyr Gly Tyr Ser Ile Asn Glu Thr Leu Ile Pro Phe Thr
 435 440 445
 Asp Ser Leu Ser Phe Lys Leu Ser Val Asp Thr Met Ser Val Leu Asn
 450 455 460
 Asp Gln Tyr Ala Lys Val Val Ile Phe Phe Asn Thr Ile Ile Glu Tyr
 465 470 475 480
 Ile Val Ala Thr Ile Tyr Tyr Arg Leu Ala Val Leu Asn Asn Tyr Asp
 485 490 495
 Gly Ile Lys His Phe Val Ser Lys Val Leu His Thr Val Ile Glu Ala
 500 505 510
 Cys Gly Val Leu Phe Ser His Ile Lys Val His Asp Lys Ile Glu His
 515 520 525
 Glu Met Glu Glu Met Val Asp Lys Gly Thr Val Pro Ser Tyr Leu Tyr
 530 535 540
 His Leu Ser Ile Thr Thr Ile Ser Ile Ile Leu Asp Asp Ile Asn Gly
 545 550 555 560
 Thr Cys