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(54) **SAFE MUTANT VIRAL VACCINES**

SICHERE, MUTIERTE VIRALE IMPFSTOFFE

VACCINS VIRAUX MUTANTS SECURISES

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- **VENUGOPAL K ET AL: "TOWARDS A NEW GENERATION OF FLAVIVIRUS VACCINES" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 12, no. 11, August 1994 (1994-08), pages 966-975, XP002048225 ISSN: 0264-410X**

Description

Field Of The Invention

[0001] The present invention relates generally to vaccines suitable for administration to animals against viral infections. More specifically, the present invention relates to safe vaccines and methods of preparing such vaccines. The vaccines of the present invention contain at least two live mutant viruses of the same family or nucleic acid molecules encoding such viruses, wherein each of the viruses or the encoding nucleic acids contains a mutation that confers a desirable phenotype and the mutations In the viruses reside in the same genomic site such that the mutant viruses cannot recombine with each other to eliminate the mutations. Specifically, two of the live mutant viruses consist of Bovine Viral Diarrhea Viruses (BVDV).

Background Of The Invention

[0002] The virus family *Flaviviridea* consists of the genera *Pestivirus*, *Flavivirus* and *Hepacivirus.* The genus *Pestivirus* is represented by the species Bovine viral diarrhea virus 1 (BVDVs-1), BVDV-2, classical swine fever virus, and Border disease virus. The virions of the family members encapsulate positive-strand RNA genomes of about 9.5 to 12.3 kb. The genomic RNAs contain contiguous long open reading frames (ORFs), which are translated into polyproteins that are processed by cellular and viral proteases to give rise to the mature viral proteins. For members of *Pestivirus,* the ORF encodes a polyprotein of about 3900 amino acids, which is cotranslationally and posttranslationally processed to the following mature viral proteins (from 5' to 3'): N^{pro}, C, E^{rns}, E1, E2, NS2-3, NS4A, NS4B, NS5A, and NS5B.

[0003] Two blotypes are found among some members of *Pestivirus* based on their effect on tissue culture cells, namely cytopathogenic (cytopathic or cp) and noncytopathogenic (noncytopathic or ncp). Genome analyses revealed insertions of cellular sequences, sometimes accompanied by duplication of viral sequences, genomic rearrangements, and/or deletions of viral sequences in the genomes of cp pestiviruses, but not in the RNAs of the corresponding ncp pestiviruses. This suggests that cp pestiviruses are evolved from ncp pestiviruses by RNA recombination.

[0004] BVDV is a widely distributed pathogen of cattle. BVDV-1 usually produces only mild diarrhea in immunocompetent animals, whereas BVDV-2 can produce thrombocytopenia, hemorrhages and acute fatal disease. BVDV is capable of crossing the placenta of pregnant cattle and may result in the birth of persistently infected (PI) calves (Malmquist, J. Am. Vet. Med. Assoc. 152:763-768 (1968); Ross, et al., J. Am. Vet. Med. Assoc. 188:618-619 (1986)). Viremic calves are Immunotolerant to the virus and persistently viremic for the rest of their lives. They provide a source for outbreaks of mucosal disease (Liess, et al., Dtsch. Tieraerztl. Wschr. 81:481-487 (1974)) and are highly predisposed to infection with microorganisms causing diseases such as pneumonia or enteric disease (Barber, et al., Vet. Rec. 117:459-464 (1985)). Viruses of either genotype may exist as one of the two biotypes, cp or ncp. The cp phenotype correlates with the expression of NS3, since cells infected with either cp or ncp BVDV both express NS2-3, whereas NS3 is detected only after infection with cp BVDV. NS3 is colinear to the C-terminal part of NS2-3. The expression of NS3 appears to be a result of genomic alterations observed for cp BVDV.

[0005] Presently available viral vaccines include killed or attenuated live viral vaccines, live-vectored vaccines, subunit vaccines, and DNA or RNA vaccines. See Roth et al., "New Technology For Improved Vaccine Safety And Efficacy", Veterinary Clinics North America: Food Animal Practice 17(3): 585-597 (2001). Attenuation of viruses can be achieved by UV irradiation, chemical treatment, or high serial passage *in vitro.* The number, position and nature of mutations induced by these methods are unknown absent genomic sequence analyses. Attenuation can also be achieved by making defined genetic alterations, for example, specific deletion of viral sequences known to confer virulence, or insertion of sequences into the viral genome. One concern with respect to the use of attenuated live viral vaccines is that attenuated mutant viruses have the potential to recombine *in vivo* to eliminate the attenuating mutation(s) thereby restoring virulence. For example, in the presence of a virulent (wild type) field strain, attenuated viruses having deletions in the viral genome have the potential to recombine with the virulent strain to restore the deleted sequence. See, e.g., Roth et al., *supra.* Cytopathic pestiviruses having cellular insertions have also been observed to give rise to noncytopathic viruses in cell culture by deletion of the cellular sequences, possibly through RNA recombination. See, e.g., Baroth et al., "Insertion of cellular NEDD8 coding sequences in a pestivirus", Virology. 278(2): 456-66, (2000), and Becher et al., "RNA recombination between persisting pestivirus and a vaccine strain: generation of cytopathogenic virus and induction of lethal disease", Journal of Virology 75(14): 6256-64 (2001). Where it is desired to include two attenuated mutant viruses from the same species, genus or family in a vaccine composition, there is a concern that the two viruses may recombine in the vaccinated animal thereby eliminating the attenuating mutations. See, e.g., Glazenburg et al., "Genetic recombination of pseudorabies virus: evidence that homologous recombination between insert sequences is less frequent than between autologous sequences", Archives of Virology, 140(4): 671-85 (1995).

[0006] There remains a need to develop safe and effective vaccines that protect animals against viral infections.

Summary Of The Invention

[0007] The present invention provides safe vaccines which contain at least two live mutant viruses of the same family or nucleic acid molecules encoding such viruses, wherein each virus or the encoding nucleic acid contains a mutation that confers a desirable phenotype, and the mutations In the viruses reside in the same genomic site such that the mutant viruses cannot recombine with each other to eliminate the mutations. Specifically, two of the live mutant viruses consist of Bovine Viral Diarrhea Viruses (BVDV).

[0008] The present invention also provides a method of preparing a safe viral vaccine by selecting or constructing two or more live mutant viruses of the same family, genus or species, wherein each virus contains a mutation that confers a desirable phenotype, and the mutations in the viruses reside in the same genomic site such that the mutant viruses can not undergo homologous recombination to eliminate the mutations. Specifically, two of the live mutant viruses consist of Bovine Viral Diarrhea Viruses (BVDV).

[0009] The present invention further provides a method of protecting an animal against viral infections by administering to the animal a vaccine composition of the present invention.

Brief Description Of The Drawings

[0010] Figure 1. Alignment of the cellular insertions and flanking viral sequences from the NS2-3 regions of BVDV-1 strain NADL and BVDV-2 strain 53637.

Detailed Description Of The Invention

[0011] It has been uniquely recognized in accordance with the present invention that live mutant viruses of the same family, which contain mutations at the same genomic site of the viruses, cannot recombine with one another to eliminate the mutations.

[0012] Accordingly, In one embodiment, the present invention provides safe vaccine compositions containing at least two, i.e., two or more, live mutant viruses of the same family, or nucleic acid molecules encoding such viruses, wherein the mutations In the viruses reside in the same genomic site such that the mutant viruses cannot recombine with each other to eliminate the mutations. Specifically, two of the live mutant viruses consist of Bovine Viral Diarrhea Viruses (BVDV).

[0013] In another embodiment, the present invention provides a method of preparing a safe viral vaccine, as described hereinabove. Specifically, a safe vaccine is prepared by selecting or constructing two or more live mutant viruses of the same family, genus or species, wherein each virus contains a mutation that confers a desirable phenotype (for example attenuation of virulence, alteration of cellular tropism or biotype, alteration of species tropism, or expression of a foreign gene cassette), and the mutations in the viruses reside in the same genomic site such that the mutant viruses can not undergo homologous recombination with each other to eliminate the mutations. Specifically, two of the live mutant viruses consist of Bovine Viral Diarrhea Viruses (BVDV).

[0014] The term "vaccine" or "vaccine composition" refers to a composition containing live mutant viruses which, upon Inoculation into an animal, Induces a complete or partial immunity to the pathogenic version of the viruses, or alleviates the symptoms of diseases caused by the pathogenic versions of the viruses. The protective effects of a vaccine composition against a virus are normally achieved by inducing in the subject an immune response, either a cell-mediated or a humoral immune response, or a combination of both. Generally speaking, abolished or reduced Incidences of viral infection, amelioration of the symptoms, or accelerated elimination of the viruses from the infected subjects, are indicative of the protective effects of the vaccine composition.

[0015] By "animal" is meant to include birds, for example, chickens, turkeys, domestic waterfowl, and any mammal, for example, cattle, sheep, swine, goats, dogs, cats, and horses.

[0016] The term "viruses", "viral isolates" or "viral strains" as used herein refer to viral particles or virions that contain viral genomic DNA or RNA, associated proteins, and other chemical constituents (such as lipids).

[0017] By "nucleic acid molecule encoding a virus" or "nucleic acid molecule of a virus" is meant the genomic nucleic acid molecule of the virus, either in the form of RNA or DNA.

[0018] By "mutation" is meant to include deletion, insertion or substitution of one or more nucleotides, or a combination thereof. In accordance with the present invention, the mutation preferably confers a desirable phenotype, for example attenuation of virulence, alteration of cellular tropism or biotype, alteration of species tropism, or expression of a foreign gene cassette. Especially preferred mutations are mutations that confer attenuated virulence.

[0019] By "attenuation" is meant that the virus has lost some or all of its ability to proliferate and/or cause disease in an animal infected with the virus. For example, an attenuated virus can be a virus that is unable to replicate at all or is limited to one or a few rounds of replication, or restricted in cell or tissue tropism, when present in an animal in which a wild type pathogenic version of the attenuated virus can replicate.

[0020] An attenuated virus may have one or more mutations in a gene or genes that are involved in pathogenicity of the virus. Such mutations are also referred to herein as "attenuating mutation(s)". An attenuated virus can be produced from the wild type, pathogenic virus by UV irradiation, chemical treatment, or high serial passage of the wild type, pathogenic virus *in vitro.* Alternatively, an attenuated virus can be produced from the wild type, pathogenic virus by making specific deletion of viral sequences known to confer virulence, insertion of sequences into the viral genome, or making one or more point mutations in the viral genome. An attenuated virus can be a viral isolate obtained from an animal, which isolate is derived from the wild type, pathogenic version of the virus through events other than artificial means, e.g., events that have occurred in a host animal such as recombination.

[0021] The two or more live mutant viruses present in the vaccine compositions of the present invention contain mutations that reside in the same genomic site. By "same genomic site" is meant that when the genomic nucleotide sequences of the viruses are aligned, the mutations in the viral genomes overlap with one another such that there is no opportunity for homologous recombination between and among the viral genomes to eliminate the mutations. In other words, when the genomic nucleotide sequences of the viruses are aligned, there is at least one contiguous portion of the aligned sequences where the sequences In the aligned viral genomes are mutant sequences. There are a number of computer programs that compare and align nucleic acid sequences which one skilled in the art may use. The sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in a nucleic acid sequence for optimal alignment with a second nucleic add sequence). For example, the NBLAST and XBLAST programs as described in Altschul, et al., 1990, J. Mol. Biol. 215:403-410, the Gapped BLAST program as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402, and the PSI-Blast program as described in Altschul et al., 1997, *supra.* When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see http://www.ncbi.nim.nih.gov).

[0022] Generally speaking, the concept of the present invention, i.e., including in the same vaccine composition two or more live mutant viruses of the same family having mutations at the same genomic site, applies to mutant viruses from any family where the viral genomes have sufficient sequence identity to permit homologous recombination. It has been shown that a nucleotide identity as short as 15 nucleotides can lead to efficient homologous recombination (Nagy and Bujarski. J. Virol. 69:131-140, 1995).

[0023] The vaccine composition of the present invention contains two or more live mutant viruses from the *Pestivirus* genus, specifically BVDV. The genus *Pestivirus* is represented by the species Bovine Viral Diarrhea Virus Type 1 (BVDV-1), Bovine Viral Diarrhea Virus Type 2 (BVDV-2), classical swine fever virus, and Border disease virus. The ORF encodes a polyprotein of about 3900 amino acids, which is co-translationally and posttranslationally processed to the following mature viral proteins (from 5' to 3'): N^{pro}, C, E^{rns}, E1, E2, NS2-3, NS4A, NS4B, NS5A, and NS5B.

[0024] Ordinarily, BVDV has a genome In the form of RNA. RNA can be reverse-transcribed into DNA for use in cloning. Thus, references made herein to nucleic acid and BVD viral sequences encompass both viral RNA sequences and DNA sequences derived from the viral RNA sequences. For convenience, genomic sequences of BVDV as depicted in the SEQUENCE LISTING hereinbelow only refer to the DNA sequences. The corresponding RNA sequence for each is readily apparent to those of skill In the art.

[0025] In a more preferred embodiment, the vaccine composition of the present invention contains a cytopathic BVDV-1 and a cytopathic BVDV-2, wherein the mutations in both viruses associated with the cytopathic biotype reside in the same genomic site such that the two mutant viruses cannot recombine to eliminate the mutations.

[0026] BVDV-1 and BVDV-2 represent two closely related genotypes of BVDV. The nucleotide sequences of the two viruses share about 70% identity over the entire genome, and slightly higher percent identity within the NS2-3 region. It is believed that the percent identity between the viral genomes of BVDV-1 and BVDV-2, at least in the NS2-3 region, is sufficient to permit homologous recombination.

[0027] BVDV-1 usually produce only mild diarrhea in animals, whereas BVDV-2 are viruses with high virulence which can produce thrombocytopenia, hemorrhages and acute fatal disease (Corapi et al., J. Virol. 63: 3934-3943; Bolin et al., Am. J. Vet. Res. 53: 2157-2163; Pellerin et al., Virology 203: 260-268, 1994; Ridpath et al., Virology 205: 66-74, 1994; Carman et al., J. Vet. Diagn. Invest. 10: 27-35, 1998). The two types of viruses have distinct antigenicity determined by a panel of MAbs and by cross-neutralization using virus-specific antisera raised in animals (Corapi et al., Am. J. Vet. Res. 51: 1388-1394, 1990). Viruses of either genotype may exist as one of the two biotypes, cytopathogenic (cytopathic or cp) or noncytopathogenic (noncytopathic or ncp). Cp viruses induce cytopathic effects (e.g., cell lysis) on cultured cells, while noncytopathic viruses do not.

[0028] It is desirable to prepare vaccines that provide protection against both BVDV-1 and BVDV-2. However, because of the high degree of sequence identity between the two viruses, there is a possibility that a live cytopathic BVDV-1 and a live cytopathic BVDV-2 included in the same vaccine composition, could recombine with each other in the vaccinated animal to yield noncytopathic viruses. Recombination between BVDV-1 and BVDV-2 has been documented. See, e.g., Ridpath et al., Virology 212: 259-262 (1995). Infection of the fetus in pregnant cattle with ncp viruses before immunocompetence develops can result in the fetus remaining viremic through the period of gestation and the subsequent birth of a calf that remains persistently viremic. Such a calf can die of mucosal disease upon superinfection with a cp BVDV.

Accordingly, the vaccine compositions provided by the present invention, which contain live cp BVDV-1 and live cp BVDV-2 having mutations at the same genomic site, are especially desirable for protecting animals against both BVDV-1 and BVDV-2.

[0029] In one embodiment, BVDV cp isolates obtained from animals can be used in the vaccine composition of the present invention. Cp isolates of both BVDV-1 and BVDV-2 have been reported and are available to those skilled in the art, e.g., BVDV-1 NADL (ATCC# VR1422 or VR-534), BVDV-2 53637 strain (deposited with the ATCC as PTA-4859), and type 2 field isolates such as those described by Ridpath and Neill, J. Virol 74:8771-8774, (2000). Cp isolates reported so far typically contain an insertion of a heterologous sequence, e.g., an ubiquitin coding sequence (Genbank accession number M96687 or De Moerlooze et al., J. Gen. Virol. 74:1433-1438, (1993)), a bovine NEDD8 coding sequence (Baroth et al., *supra*), or a *Bos taurus* DnaJ1 coding sequence (as described in the Examples hereinbelow), among others.

[0030] In another embodiment, a cp BVDV is generated by making defined alterations in the BVDV genome, e.g., by deleting specific viral sequences, inserting sequences into a specific viral genomic site, or making one or more substitutions, or combinations thereof.

[0031] Where a cp BVDV is generated by inserting a heterologous (i.e., foreign to the virus) sequence into a specific genomic site, the nature of the sequence to be inserted is generally not critical to the present invention. In addition, the insertion is not limited to any particular site so long as the insertion results in an attenuated phenotype. As heterologous sequences in cp isolates are often found in the NS2-3 region, the NS2-3 region, especially the part surrounding the putative NS2-3 cleavage site which corresponds to, e.g., amino acid residues # 1679 to #1680 of the BVDV-1 NADL strain (the numbering is based on the published genomic sequence Genbank accession No. M31182, SEQ ID NO: 4), is a preferred location for insertions.

[0032] An cp BVDV-1 can be generated by making a defined genomic alteration that mimics the mutation identified in a cp BVDV-2 isolate obtained from an animal, such that these viruses have mutations associated with the cp biotype in the same genomic site. Similarly, a cp BVDV-2 can be generated by way of making a defined genomic alteration that mimics the mutation identified in a cp BVDV-1 isolate obtained from an animal.

[0033] In a preferred embodiment, the vaccine composition of the present invention contains NADL (a cp BVDV-1 isolate), and BVDV-2 53637 (a cp BVDV-2 isolate), where the two cp isolates each contain a mutation at the same genomic site which results in the cytopathic biotype. The genomic sequence of the BVDV-1 NADL strain is set forth in SEQ ID NO: 4, and the BVDV-2 53637 strain was deposited with the ATCC as PTA-4859. Both isolates contain an insertion in the NS2-3 region. The attenuated cp BVDV-1 contains an insertion of a *Bos taurus* DnaJ1 coding sequence 3' of the thymidine at nucleotide position # 4993 (NADL sequence numbering), which is the third nucleotide of the codon encoding the glycine residue at amino acid position 1536. The attenuated cp BVDV-2 contains an insertion of a Bos *taurus* DnaJ1 coding sequence at the same genomic site.

[0034] According to the present invention, the cp BVDV isolates employed in the present vaccine composition have been attenuated and are therefore nonpathogenic. Methods of attenuation are known to those skilled in the art and are also described hereinbelow.

[0035] In another embodiment, the vaccine composition of the present invention contains an attenuated BVDV-1 and an attenuated BVDV-2, wherein the attenuating mutations in both viruses reside in the same genomic site such that the two mutant viruses cannot recombine to eliminate the attenuating mutations.

[0036] An attenuated BVDV is generated by UV irradiation, chemical treatment, or high serial passage of the pathogenic version of the viruse *in vitro.* Sequence analysis can be conducted in order to determine the nature and genomic location of mutations generated by these methods. The mutation can be in the form of a deletion, insertion or substitution of one or more nucleotides, or a combination thereof. Alternatively, an attenuated BVDV is generated by making defined alterations in the BVDV genome, e.g., by deleting specific viral sequences, inserting sequences into a specific viral genomic site, or making one or more substitutions, or combinations thereof.

[0037] As described above, the live mutant viruses for use in the vaccine composition of the present invention can be from the same family, genus or species, where the viral genomes have sufficient sequence identity to permit homologous recombination. Additional examples of combinations of viruses appropriate for use in the vaccine composition of the present invention Include, but are not limited to, combinations of different types of pollovirus, combinations of multiple live mutant strains of infectious bronchitis virus, combinations of multiple live mutant strains of Newcastle disease virus, combinations of Canine adenovirus - 1 and canine adenovirus-2, combinations of equine herpesvirus-1 and equine herpesvirus-4, combinations of multiple live mutant strains of influenza virus, combinations of multiple live attenuated strains of Feline calicivirus, combinations of multiple serotypes of Rotavirus, combinations of multiple serotypes of Rhinovirus, combinations of multiple serotypes of Foot and Mouth Disease virus, combinations of the European and North American genotypes of Porcine reproductive and respiratory syndrome virus, combinations of standard and variant strains of infectious bursal disease virus.

[0038] In accordance with the present invention, although viral particles are the preferred form for use in the vaccines, nucleic add molecules encoding mutant viruses of the same family, genus or species, can be used directly in vaccines as well. The DNA or RNA molecule can be present in a "naked' form or it can be combined with an agent which facilitates cellular uptake (e.g., liposomes or cationic lipids). Vaccines and vaccination procedures that utilize nucleic acids (DNA or mRNA) have been well described in the art, e.g., U.S. Patent No. 5,703,055, U.S. Patent No. 5,580,859, U.S. Patent No. 5,589,466, international Patent Publication WO 98/35562, and by Ramsay et al., 1997, Immunol. Cell Biol. 75: 360-363; Davis, 1997, Cur. Opinion Biotech. 8: 635-640; Manickan et al., 1997, Critical Rev. Immunol. 17: 139-154; Robinson, 1997, Vaccine 15(8): 785-787; Robinson et al., 1996, AIDS Res. Hum. Retr. 12(5): 455-457; Lal and Bennett, 1998, Critical Rev. Immunol. 18:449-484; and Vogel and Sarver, 1995, Clin. Microbiol. Rev. 8(3): 406-410, all of which are incorporated herein by reference.

[0039] In addition to two or more live mutant viruses from the same family, genus or species (specifically BVDV), the vaccine compositions can include other antigenic component. Other antigenic components appropriate for use in accordance with the present invention Include, but are not limited to, antigens prepared from pathogenic bacteria such as *Mycoplasma hyopneumonia, Haemophilus somnus, Haemophilus parasuls, Bordetella bronchiseptica, Bacillus anthracis, Actinobacillus pleuropneumonie, Pasteurella multocida, Mannhemia haemolytica, Mycoplasma bovis, Mycoplasma galanacieum, Mycoplasma gallisepticum, Mycobacterium bovis*, *Mycobacterium paratuberculosis, Clostridial spp., Streptococcus uberis, Streptococcus suis, Staphylococcus aureus, Erysipelothrix rhusopathiae, Campylobacter spp., Fusobacterium necrophorum, Escherichia coli, Lawsonia intracellularis, Listeria monocytogenes, Rickettsia rickettsii, Borrelia spp., Ehrlichia spp., Chlamydia spp., Brucella spp., Vibrio spp., Salmonella enterica* serovars, *Leptospira spp.*; pathogenic fungi such as *Candida*; protozoa such as *Cryptosporidium parvum, Neospora canium, Toxoplasma gondii, Eimeria spp., Babesia spp., Giardia spp.;* helminths such as *Ostertagia, Cooperia, Haemonchus, Fasciola*; either in the form of an inactivated whole or partial cell preparation, or in the form of antigenic molecules obtained by genetic engineering techniques or chemical synthesis. Additional antigens include pathogenic viruses such as Marek's disease virus, infectious bursal disease virus, Newcastle's disease virus, chicken anemia virus, fowlpox virus, avian leukosis virus, infectious laryngotracheitis virus, reticuloendothelial virus, canine parvovirus, canine distemper virus, canine herpesvirus, canine coronavirus, canine parainfluenza-5, feline panleukopenia virus, feline herpes virus, feline calicivirus, feline immunodeficiency virus, feline infectious peritonitis virus, equine herpesvirus, equine arteritis virus, equine infectious anemia virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, West Nile virus, transmissible gastroenteritis virus, bovine coronavirus, Bovine herpesviruses-1,3,6, Bovine parainfluenza virus, Bovine respiratory syncytial virus, bovine leukosis virus, rinderpest virus, foot and mouth disease virus, rabies virus, African swine fever virus, Porcine parvovirus, PRRS virus, Porcine circovirus, influenza virus, swine vesicular disease virus, Techen fever virus, Pseudorabies virus, either in the form of modified live (attenuated) viral preparation, an inactivated whole or partial virus preparation, or in the form of antigenic molecules obtained by genetic engineering techniques or chemical synthesis. When additional attenuated live viruses are used, such additional viruses should preferably be from a family different from that of the two principal attenuated viruses, as described above.

[0040] In a preferred embodiment, the present invention provides a vaccine composition which contains an attenuated cp BVDV-1 derived from the BVDV-1 NADL strain, an attenuated cp BVDV-2 derived from the BVDV-2 53637 strain, where the two cp isolates each contain a mutation associated with the cp biotype at the same genomic site, and at least one (i.e., one or more) of the following antigenic component, either in inactivated or modified live form: bovine herpesvirus-1, bovine respiratory syncytial virus, parainfluenza virus-3, *Campylobacter fetus, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagiae, Leptospira pomona,* or *Mannhemia haemolytica.*

[0041] In addition, the vaccine compositions of the present invention can include one or more veterinarily-acceptable carriers. As used herein, "a veterinarily-acceptable carrier" includes any and all solvents, dispersion media, coatings, adjuvants, stabilizing agents, diluents, preservatives, antibacterial and antifungal agents, isotonic agents, adsorption delaying agents, and the like. Diluents can include water, saline, dextrose, ethanol, glycerol, and the like. Isotonic agents can include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin, among others. The vaccine compositions can further include one or more other immunomodulatory agents such as, e.g., interleukins, interferons, or other cytokines

[0042] Adjuvants suitable for use in the vaccine compositions include, but are not limited to, the RIBI adjuvant system (Ribi inc.), alum, aluminum hydroxide gel, oil-in water emulsions, water-in-oll emulsions such as, e.g., Freund's complete and incomplete adjuvants, Block co polymer (CytRx, Atlanta GA), SAF-M (Chiron, Emeryville CA), AMPHIGEN® adjuvant, saponin, Quil A, cholesterol, QS-21 (Cambridge Biotech Inc., Cambridge MA), or other saponin fractions, monophosphoryl lipid A, Avridine lipid-amine adjuvant, heat-labile enterotoxin from *E. coli* (recombinant or otherwise), cholera toxin, or muramyl dipeptide, among many others.

[0043] Typically, a live mutant virus is present in a vaccine at an amount of about 1 x 10^6 and about 1 x 10^8 virus particles per dose, with a veterinarily acceptable carrier, in a volume of between about 0.5 and about 5 ml. The precise amount of a virus in a vaccine composition effective to provide a protective effect can be determined by a skilled veterinarian. Where the DNA or RNA molecule of the virus is used in the vaccine, the amount of the nucleic acids should generally be between about 0.1 μg/ml and about 5.0 mg/ml.

[0044] The vaccine compositions of the present invention can be made in various forms depending upon the route of administration. For example, the vaccine compositions can be made in the form of sterile aqueous solutions or dispersions suitable for injectable use, or made in lyophilized forms using freeze-drying techniques. Lyophilized compositions are typically maintained at about 4°C, and can be reconstituted in a stabilizing solution, e.g., saline or and HEPES, with or without adjuvant

[0045] The vaccine compositions of the present invention can be administered to an animal for treating or preventing a disease caused by the pathogenic versions of the viruses in the vaccine compositions. Therefore, methods of vaccinating an animal against a disease caused by a virus are also dercribed.

[0046] In practicing the present methods, a vaccine composition of the present invention is administered to an animal preferably via parenteral routes, although other routes of administration can be used as well, such as e.g., by oral, intranasal, intramuscular, intra-lymph node, intradermal, intraperitoneal, subcutaneous, rectal or vaginal administration, or by a combination of routes. Boosting regimens may be required and the dosage regimen can be adjusted to provide optimal vaccination.

[0047] The present invention is further illustrated by, but by no means limited to, the following examples.

EXAMPLE I

Determination Of The Position Of The Cellular Insertion In BVDV2 Strain 53637

[0048] A portion of the sequence of the NS2-3 region from BVDV2-53637 was determined, in order to identify and map the location of any cellular insertions in the region. A 670 base RT-PCR product was amplified from viral RNA, using forward primer 53637U1 (5'-CGTCCACAGATGGTTTGGT-3'; SEQ ID NO: 1) and reverse primer 53637L (5'-GGCTATGTATTGGACGTAACCC-3'; SEQ ID NO: 2). The RT-PCR product was purified and submitted for sequence analysis (SEQ ID NO: 3). When aligned with BVDV1-NADL (Genbank accession number M31182, SEQ ID NO: 4), striking similarities were observed **(FIGURE 1).** Both viruses contain an in-frame insertion derived from the *Bos taurus Dna*J1 gene. In the case of NADL, the insertion is 90 amino acids (270 nucleotides) in length and is located between glycine-1536 and proline-1627 in the NADL polyprotein. These coordinates correspond to glycine-1536 and proline-1537 in non-cytopathic BVDV1 strains such as SD-1 (Genbank accession number AAA42860, SEQ ID NO: 6), indicating that the genome alteration in NADL is a simple insertion with no concomitant deletion or duplication of flanking viral sequences. Like BVDV1-NADL, there is an insertion of a portion of the *Bos taurus Dna*J1 gene in BVDV2-53637. The cellular insertion is longer (131 amino acids, 393 nucleotides), being extended in both directions relative to the insertion in BVDV1-NADL. The location of the cellular insertion within the NS2-3 region is identical in the two viruses. Unlike BVDV1-NADL, the BVDV2-53637 insertion is accompanied by a deletion of 5 amino acids (15 nucleotides) of flanking viral sequences. Three amino acid residues are absent flanking the 5' end of the insertion, while two amino acids residues are absent flanking the 3' end of the insertion. Because the cellular insertions are at the same genome position in the two vaccine viruses, they cannot undergo homologous recombination to delete the insertion to generate a non-cytopathic chimeric virus.

Example II

Attempts To Detect Non-Cytopathic BVDV Viruses In Co-Passaged BVDV1-NADL / BVDV2-53637 Cultures

[0049] In order to determine whether the two vaccine viruses are capable of recombining to generate detectable levels of non-cytopathic BVDV, the viruses were co-cultivated on susceptible cells and a sensitive hemi-nested RT-PCR assay was used to detect potential non-cytopathic viruses from among an excess of longer cytopathic products that still contain the cellular insert. To increase the probability of intertypic recombination *in vitro,* each virus was inoculated simultaneously onto confluent BK-6 cells in 6-well plates at a multiplicity of infection of 2-4 (12 replicates per experiment). After 2 - 3 days of co-cultivation the cells were frozen and thawed twice, and cell debris was removed by low speed centrifugation. The resulting supernatant fluid was then used as inoculum for the next passage. A total of seven serial passages were conducted in several studies. During the passages BVDV1-NADL grew more rapidly than BVDV2-53637, but the type II virus was still detectable after seven passages using nested RT-PCR. A sensitive hemi-nested RT-PCR assay was employed in an attempt to detect any non-cytopathic virus.

[0050] In first round RT-PCR, forward primers 53637U1 (SEQ ID NO: 1) or NADL4744 (5'-CGTGGCTTCTTGGTACG-GG-3', SEQ ID NO: 7) were used in conjunction with reverse primers 53637L (SEQ ID NO: 2) or NADL5305 (5'- AGCG-GTATATTGTACAAAGCCA-3', SEQ IDNO: 8). All four combinations of forward and reverse primers were used in order to detect BVDV1, BVDV2, and intertypic recombinants. The expected size of RT-PCR product was 562 bp for cytopathic BVDV1-NADL and 670 bp for cytopathic BVDV2-53637. Non-cytopathic viruses, if present at detectible levels, would be expected to yield first round products of 292 bp (BVDV1-NADL) or 277 bp (BVDV2-53637). Intertypic recombinants should be similar in size to one of the parents, or of intermediate length, depending on the location of the recombination site. Non-cytopathic BVDVs were never detected following first round RT-PCR.

[0051] To increase the sensitivity of detecting non-cytopathic BVDV in the presence of a large excess of cytopathic BVDV, a restriction enzyme digestion step was included before the nested PCR to destroy the larger NS2-3 templates derived from the cytopathic viruses. A combination of *Msp*I and *Dra*I was selected based on the observation that they cut within the *Bos taurus Dna*J1 insert but do not cut the flanking viral sequences. In second round (hemi-nested) PCR, forward primers 53637U2 (5'-TGCACGATCTGTGAAGGGAAAGAA -3', SEQ ID NO: 9) or NADL4844 (5'- TGCACTG-TATGTGAGGGCCGAGAG -3', SEQ ID NO: 10) were used in conjunction with the same two reverse primers 53637L or NADL5305. Appropriate primer combinations were used to attempt to detect intertypic recombinants as well as BVDV1 and BVDV2. The expected size of RT-PCR product is 462 bp for cytopathic BVDV1-NADL and 570 bp for cytopathic BVDV2-53637 (present at low levels due to incomplete digestion of the cytopathic BVDV RT-PCR products). Non-cytopathic viruses, if present at detectable levels, would be expected to yield second round products of 192 bp (BVDV1-NADL) or 177 bp (BVDV2-53637). Intertypic recombinants should be similar in size to one of the parents, or of intermediate length, depending on the location of the recombination site. Non-cytopathic BVDVs were never detected following second round PCR. In a few individual reactions, aberrant bands of various sizes were seen. All bands between 100 and 300 bp were considered to be potential non-cytopathic products and were submitted for DNA sequence analysis. In every case the aberrant band was the result of false priming during PCR. There was no evidence of non-cytopathic virus in any of the studies.

SEQUENCE LISTING

[0052]

<110> Welch, Siao-Kun Wan, et al.

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```
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<400> 5

```
Met Glu Leu Ile Thr Asn Glu Leu Leu Tyr Lys Thr Tyr Lys Gln Lys
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Pro Val Gly Val Glu Glu Pro Val Tyr Asp Gln Ala Gly Asp Pro Leu
            20                  25                  30
```

```
Phe Gly Glu Arg Gly Ala Val His Pro Gln Ser Thr Leu Lys Leu Pro
        35                  40                  45

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

Lys Arg Gly Asp Cys Arg Ser Gly Asn Ser Arg Gly Pro Val Ser Gly
65                  70                  75                  80

Ile Tyr Leu Lys Pro Gly Pro Leu Phe Tyr Gln Asp Tyr Lys Gly Pro
                85                  90                  95

Val Tyr His Arg Ala Pro Leu Glu Leu Phe Glu Glu Gly Ser Met Cys
            100                 105                 110

Glu Thr Thr Lys Arg Ile Gly Arg Val Thr Gly Ser Asp Gly Lys Leu
        115                 120                 125

Tyr His Ile Tyr Val Cys Ile Asp Gly Cys Ile Ile Ile Lys Ser Ala
    130                 135                 140

Thr Arg Ser Tyr Gln Arg Val Phe Arg Trp Val His Asn Arg Leu Asp
145                 150                 155                 160

Cys Pro Leu Trp Val Thr Thr Cys Ser Asp Thr Lys Glu Glu Gly Ala
                165                 170                 175

Thr Lys Lys Lys Thr Gln Lys Pro Asp Arg Leu Glu Arg Gly Lys Met
            180                 185                 190

Lys Ile Val Pro Lys Glu Ser Glu Lys Asp Ser Lys Thr Lys Pro Pro
        195                 200                 205

Asp Ala Thr Ile Val Val Glu Gly Val Lys Tyr Gln Val Arg Lys Lys
    210                 215                 220

Gly Lys Thr Lys Ser Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys
225                 230                 235                 240

Asn Lys Pro Gln Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala
                245                 250                 255

Trp Ala Ile Ile Ala Ile Val Leu Phe Gln Val Thr Met Gly Glu Asn
            260                 265                 270

Ile Thr Gln Trp Asn Leu Gln Asp Asn Gly Thr Glu Gly Ile Gln Arg
        275                 280                 285
```

```
Ala Met Phe Gln Arg Gly Val Asn Arg Ser Leu His Gly Ile Trp Pro
    290                 295                 300

Glu Lys Ile Cys Thr Gly Val Pro Ser His Leu Ala Thr Asp Ile Glu
305                 310                 315                 320

Leu Lys Thr Ile His Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr
                325                 330                 335

Thr Cys Cys Arg Leu Gln Arg His Glu Trp Asn Lys His Gly Trp Cys
            340                 345                 350

Asn Trp Tyr Asn Ile Glu Pro Trp Ile Leu Val Met Asn Arg Thr Gln
        355                 360                 365

Ala Asn Leu Thr Glu Gly Gln Pro Pro Arg Glu Cys Ala Val Thr Cys
    370                 375                 380

Arg Tyr Asp Arg Ala Ser Asp Leu Asn Val Val Thr Gln Ala Arg Asp
385                 390                 395                 400

Ser Pro Thr Pro Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe
                405                 410                 415

Ala Gly Ile Leu Met Arg Gly Pro Cys Asn Phe Glu Ile Ala Ala Ser
            420                 425                 430

Asp Val Leu Phe Lys Glu His Glu Arg Ile Ser Met Phe Gln Asp Thr
        435                 440                 445

Thr Leu Tyr Leu Val Asp Gly Leu Thr Asn Ser Leu Glu Gly Ala Arg
    450                 455                 460

Gln Gly Thr Ala Lys Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile
465                 470                 475                 480

Leu Gly Lys Lys Leu Glu Asn Lys Ser Lys Thr Trp Phe Gly Ala Tyr
                485                 490                 495

Ala Ala Ser Pro Tyr Cys Asp Val Asp Arg Lys Ile Gly Tyr Ile Trp
            500                 505                 510

Tyr Thr Lys Asn Cys Thr Pro Ala Cys Leu Pro Lys Asn Thr Lys Ile
        515                 520                 525

Val Gly Pro Gly Lys Phe Gly Thr Asn Ala Glu Asp Gly Lys Ile Leu
    530                 535                 540
```

```
His Glu Met Gly Gly His Leu Ser Glu Val Leu Leu Leu Ser Leu Val
545                 550                 555                 560

Val Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Val Met Tyr Leu Ile
                565                 570                 575

Leu His Phe Ser Ile Pro Gln Ser His Val Asp Val Met Asp Cys Asp
            580                 585                 590

Lys Thr Gln Leu Asn Leu Thr Val Glu Leu Thr Thr Ala Glu Val Ile
        595                 600                 605

Pro Gly Ser Val Trp Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asn
    610                 615                 620

Trp Trp Pro Tyr Glu Thr Thr Val Val Leu Ala Phe Glu Glu Val Ser
625                 630                 635                 640

Gln Val Val Lys Leu Val Leu Arg Ala Leu Arg Asp Leu Thr Arg Ile
                645                 650                 655

Trp Asn Ala Ala Thr Thr Thr Ala Phe Leu Val Cys Leu Val Lys Ile
            660                 665                 670

Val Arg Gly Gln Met Val Gln Gly Ile Leu Trp Leu Leu Leu Ile Thr
        675                 680                 685

Gly Val Gln Gly His Leu Asp Cys Lys Pro Glu Phe Ser Tyr Ala Ile
    690                 695                 700

Ala Lys Asp Glu Arg Ile Gly Gln Leu Gly Ala Glu Gly Leu Thr Thr
705                 710                 715                 720

Thr Trp Lys Glu Tyr Ser Pro Gly Met Lys Leu Glu Asp Thr Met Val
                725                 730                 735

Ile Ala Trp Cys Glu Asp Gly Lys Leu Met Tyr Leu Gln Arg Cys Thr
            740                 745                 750

Arg Glu Thr Arg Tyr Leu Ala Ile Leu His Thr Arg Ala Leu Pro Thr
        755                 760                 765

Ser Val Val Phe Lys Lys Leu Phe Asp Gly Arg Lys Gln Glu Asp Val
    770                 775                 780

Val Glu Met Asn Asp Asn Phe Glu Phe Gly Leu Cys Pro Cys Asp Ala
785                 790                 795                 800
```

```
Lys Pro Ile Val Arg Gly Lys Phe Asn Thr Thr Leu Leu Asn Gly Pro
                805                 810                 815

Ala Phe Gln Met Val Cys Pro Ile Gly Trp Thr Gly Thr Val Ser Cys
            820                 825                 830

Thr Ser Phe Asn Met Asp Thr Leu Ala Thr Thr Val Val Arg Thr Tyr
        835                 840                 845

Arg Arg Ser Lys Pro Phe Pro His Arg Gln Gly Cys Ile Thr Gln Lys
    850                 855                 860

Asn Leu Gly Glu Asp Leu His Asn Cys Ile Leu Gly Gly Asn Trp Thr
865                 870                 875                 880

Cys Val Pro Gly Asp Gln Leu Leu Tyr Lys Gly Gly Ser Ile Glu Ser
                885                 890                 895

Cys Lys Trp Cys Gly Tyr Gln Phe Lys Glu Ser Glu Gly Leu Pro His
            900                 905                 910

Tyr Pro Ile Gly Lys Cys Lys Leu Glu Asn Glu Thr Gly Tyr Arg Leu
        915                 920                 925

Val Asp Ser Thr Ser Cys Asn Arg Glu Gly Val Ala Ile Val Pro Gln
    930                 935                 940

Gly Thr Leu Lys Cys Lys Ile Gly Lys Thr Thr Val Gln Val Ile Ala
945                 950                 955                 960

Met Asp Thr Lys Leu Gly Pro Met Pro Cys Arg Pro Tyr Glu Ile Ile
                965                 970                 975

Ser Ser Glu Gly Pro Val Glu Lys Thr Ala Cys Thr Phe Asn Tyr Thr
            980                 985                 990

Lys Thr Leu Lys Asn Lys Tyr Phe  Glu Pro Arg Asp Ser  Tyr Phe Gln
        995                 1000                 1005

Gln Tyr  Met Leu Lys Gly Glu  Tyr Gln Tyr Trp Phe  Asp Leu Glu
    1010                 1015                 1020

Val Thr  Asp His His Arg Asp  Tyr Phe Ala Glu Ser  Ile Leu Val
    1025                 1030                 1035

Val Val  Val Ala Leu Leu Gly  Gly Arg Tyr Val Leu  Trp Leu Leu
    1040                 1045                 1050
```

```
Val Thr Tyr Met Val Leu Ser Glu Gln Lys Ala Leu Gly Ile Gln
    1055                1060                1065

Tyr Gly Ser Gly Glu Val Val Met Met Gly Asn Leu Leu Thr His
    1070                1075                1080

Asn Asn Ile Glu Val Val Thr Tyr Phe Leu Leu Leu Tyr Leu Leu
    1085                1090                1095

Leu Arg Glu Glu Ser Val Lys Lys Trp Val Leu Leu Leu Tyr His
    1100                1105                1110

Ile Leu Val Val His Pro Ile Lys Ser Val Ile Val Ile Leu Leu
    1115                1120                1125

Met Ile Gly Asp Val Val Lys Ala Asp Ser Gly Gly Gln Glu Tyr
    1130                1135                1140

Leu Gly Lys Ile Asp Leu Cys Phe Thr Thr Val Val Leu Ile Val
    1145                1150                1155

Ile Gly Leu Ile Ile Ala Arg Arg Asp Pro Thr Ile Val Pro Leu
    1160                1165                1170

Val Thr Ile Met Ala Ala Leu Arg Val Thr Glu Leu Thr His Gln
    1175                1180                1185

Pro Gly Val Asp Ile Ala Val Ala Val Met Thr Ile Thr Leu Leu
    1190                1195                1200

Met Val Ser Tyr Val Thr Asp Tyr Phe Arg Tyr Lys Lys Trp Leu
    1205                1210                1215

Gln Cys Ile Leu Ser Leu Val Ser Ala Val Phe Leu Ile Arg Ser
    1220                1225                1230

Leu Ile Tyr Leu Gly Arg Ile Glu Met Pro Glu Val Thr Ile Pro
    1235                1240                1245

Asn Trp Arg Pro Leu Thr Leu Ile Leu Leu Tyr Leu Ile Ser Thr
    1250                1255                1260

Thr Ile Val Thr Arg Trp Lys Val Asp Val Ala Gly Leu Leu Leu
    1265                1270                1275

Gln Cys Val Pro Ile Leu Leu Leu Val Thr Thr Leu Trp Ala Asp
    1280                1285                1290
```

```
Phe Leu  Thr Leu Ile Leu Ile  Leu Pro Thr Tyr Glu  Leu Val Lys
    1295                 1300                 1305

Leu Tyr  Tyr Leu Lys Thr Val  Arg Thr Asp Thr Glu  Arg Ser Trp
    1310                 1315                 1320

Leu Gly  Gly Ile Asp Tyr Thr  Arg Val Asp Ser Ile  Tyr Asp Val
    1325                 1330                 1335

Asp Glu  Ser Gly Glu Gly Val  Tyr Leu Phe Pro Ser  Arg Gln Lys
    1340                 1345                 1350

Ala Gln  Gly Asn Phe Ser Ile  Leu Leu Pro Leu Ile  Lys Ala Thr
    1355                 1360                 1365

Leu Ile  Ser Cys Val Ser Ser  Lys Trp Gln Leu Ile  Tyr Met Ser
    1370                 1375                 1380

Tyr Leu  Thr Leu Asp Phe Met  Tyr Tyr Met His Arg  Lys Val Ile
    1385                 1390                 1395

Glu Glu  Ile Ser Gly Gly Thr  Asn Ile Ile Ser Arg  Leu Val Ala
    1400                 1405                 1410

Ala Leu  Ile Glu Leu Asn Trp  Ser Met Glu Glu Glu  Glu Ser Lys
    1415                 1420                 1425

Gly Leu  Lys Lys Phe Tyr Leu  Leu Ser Gly Arg Leu  Arg Asn Leu
    1430                 1435                 1440

Ile Ile  Lys His Lys Val Arg  Asn Glu Thr Val Ala  Ser Trp Tyr
    1445                 1450                 1455

Gly Glu  Glu Glu Val Tyr Gly  Met Pro Lys Ile Met  Thr Ile Ile
    1460                 1465                 1470

Lys Ala  Ser Thr Leu Ser Lys  Ser Arg His Cys Ile  Ile Cys Thr
    1475                 1480                 1485

Val Cys  Glu Gly Arg Glu Trp  Lys Gly Gly Thr Cys  Pro Lys Cys
    1490                 1495                 1500

Gly Arg  His Gly Lys Pro Ile  Thr Cys Gly Met Ser  Leu Ala Asp
    1505                 1510                 1515

Phe Glu  Glu Arg His Tyr Lys  Arg Ile Phe Ile Arg  Glu Gly Asn
    1520                 1525                 1530
```

```
Phe Glu Gly Met Cys Ser Arg Cys Gln Gly Lys His Arg Arg Phe
    1535                1540                1545

Glu Met Asp Arg Glu Pro Lys Ser Ala Arg Tyr Cys Ala Glu Cys
    1550                1555                1560

Asn Arg Leu His Pro Ala Glu Glu Gly Asp Phe Trp Ala Glu Ser
    1565                1570                1575

Ser Met Leu Gly Leu Lys Ile Thr Tyr Phe Ala Leu Met Asp Gly
    1580                1585                1590

Lys Val Tyr Asp Ile Thr Glu Trp Ala Gly Cys Gln Arg Val Gly
    1595                1600                1605

Ile Ser Pro Asp Thr His Arg Val Pro Cys His Ile Ser Phe Gly
    1610                1615                1620

Ser Arg Met Pro Phe Arg Gln Glu Tyr Asn Gly Phe Val Gln Tyr
    1625                1630                1635

Thr Ala Arg Gly Gln Leu Phe Leu Arg Asn Leu Pro Val Leu Ala
    1640                1645                1650

Thr Lys Val Lys Met Leu Met Val Gly Asn Leu Gly Glu Glu Ile
    1655                1660                1665

Gly Asn Leu Glu His Leu Gly Trp Ile Leu Arg Gly Pro Ala Val
    1670                1675                1680

Cys Lys Lys Ile Thr Glu His Glu Lys Cys His Ile Asn Ile Leu
    1685                1690                1695

Asp Lys Leu Thr Ala Phe Phe Gly Ile Met Pro Arg Gly Thr Thr
    1700                1705                1710

Pro Arg Ala Pro Val Arg Phe Pro Thr Ser Leu Leu Lys Val Arg
    1715                1720                1725

Arg Gly Leu Glu Thr Ala Trp Ala Tyr Thr His Gln Gly Gly Ile
    1730                1735                1740

Ser Ser Val Asp His Val Thr Ala Gly Lys Asp Leu Leu Val Cys
    1745                1750                1755

Asp Ser Met Gly Arg Thr Arg Val Val Cys Gln Ser Asn Asn Arg
    1760                1765                1770
```

```
Leu Thr  Asp Glu Thr Glu Tyr  Gly Val Lys Thr Asp  Ser Gly Cys
    1775                 1780                 1785

Pro Asp  Gly Ala Arg Cys Tyr  Val Leu Asn Pro Glu  Ala Val Asn
    1790                 1795                 1800

Ile Ser  Gly Ser Lys Gly Ala  Val Val His Leu Gln  Lys Thr Gly
    1805                 1810                 1815

Gly Glu  Phe Thr Cys Val Thr  Ala Ser Gly Thr Pro  Ala Phe Phe
    1820                 1825                 1830

Asp Leu  Lys Asn Leu Lys Gly  Trp Ser Gly Leu Pro  Ile Phe Glu
    1835                 1840                 1845

Ala Ser  Ser Gly Arg Val Val  Gly Arg Val Lys Val  Gly Lys Asn
    1850                 1855                 1860

Glu Glu  Ser Lys Pro Thr Lys  Ile Met Ser Gly Ile  Gln Thr Val
    1865                 1870                 1875

Ser Lys  Asn Arg Ala Asp Leu  Thr Glu Met Val Lys  Lys Ile Thr
    1880                 1885                 1890

Ser Met  Asn Arg Gly Asp Phe  Lys Gln Ile Thr Leu  Ala Thr Gly
    1895                 1900                 1905

Ala Gly  Lys Thr Thr Glu Leu  Pro Lys Ala Val Ile  Glu Glu Ile
    1910                 1915                 1920

Gly Arg  His Lys Arg Val Leu  Val Leu Ile Pro Leu  Arg Ala Ala
    1925                 1930                 1935

Ala Glu  Ser Val Tyr Gln Tyr  Met Arg Leu Lys His  Pro Ser Ile
    1940                 1945                 1950

Ser Phe  Asn Leu Arg Ile Gly  Asp Met Lys Glu Gly  Asp Met Ala
    1955                 1960                 1965

Thr Gly  Ile Thr Tyr Ala Ser  Tyr Gly Tyr Phe Cys  Gln Met Pro
    1970                 1975                 1980

Gln Pro  Lys Leu Arg Ala Ala  Met Val Glu Tyr Ser  Tyr Ile Phe
    1985                 1990                 1995

Leu Asp  Glu Tyr His Cys Ala  Thr Pro Glu Gln Leu  Ala Ile Ile
    2000                 2005                 2010
```

```
Gly Lys  Ile His Arg Phe Ser  Glu Ser Ile Arg Val  Val Ala Met
    2015                 2020                 2025

Thr Ala  Thr Pro Ala Gly Ser  Val Thr Thr Thr Gly  Gln Lys His
    2030                 2035                 2040

Pro Ile  Glu Glu Phe Ile Ala  Pro Glu Val Met Lys  Gly Glu Asp
    2045                 2050                 2055

Leu Gly  Ser Gln Phe Leu Asp  Ile Ala Gly Leu Lys  Ile Pro Val
    2060                 2065                 2070

Asp Glu  Met Lys Gly Asn Met  Leu Val Phe Val Pro  Thr Arg Asn
    2075                 2080                 2085

Met Ala  Val Glu Val Ala Lys  Lys Leu Lys Ala Lys  Gly Tyr Asn
    2090                 2095                 2100

Ser Gly  Tyr Tyr Tyr Ser Gly  Glu Asp Pro Ala Asn  Leu Arg Val
    2105                 2110                 2115

Val Thr  Ser Gln Ser Pro Tyr  Val Ile Val Ala Thr  Asn Ala Ile
    2120                 2125                 2130

Glu Ser  Gly Val Thr Leu Pro  Asp Leu Asp Thr Val  Ile Asp Thr
    2135                 2140                 2145

Gly Leu  Lys Cys Glu Lys Arg  Val Arg Val Ser Ser  Lys Ile Pro
    2150                 2155                 2160

Phe Ile  Val Thr Gly Leu Lys  Arg Met Ala Val Thr  Val Gly Glu
    2165                 2170                 2175

Gln Ala  Gln Arg Arg Gly Arg  Val Gly Arg Val Lys  Pro Gly Arg
    2180                 2185                 2190

Tyr Tyr  Arg Ser Gln Glu Thr  Ala Thr Gly Ser Lys  Asp Tyr His
    2195                 2200                 2205

Tyr Asp  Leu Leu Gln Ala Gln  Arg Tyr Gly Ile Glu  Asp Gly Ile
    2210                 2215                 2220

Asn Val  Thr Lys Ser Phe Arg  Glu Met Asn Tyr Asp  Trp Ser Leu
    2225                 2230                 2235

Tyr Glu  Glu Asp Ser Leu Leu  Ile Thr Gln Leu Glu  Ile Leu Asn
    2240                 2245                 2250
```

```
Asn Leu  Leu Ile Ser Glu Asp  Leu Pro Ala Ala Val  Lys Asn Ile
    2255                 2260                 2265

Met Ala  Arg Thr Asp His Pro  Glu Pro Ile Gln Leu  Ala Tyr Asn
    2270                 2275                 2280

Ser Tyr  Glu Val Gln Val Pro  Val Leu Phe Pro Lys  Ile Arg Asn
    2285                 2290                 2295

Gly Glu  Val Thr Asp Thr Tyr  Glu Asn Tyr Ser Phe  Leu Asn Ala
    2300                 2305                 2310

Arg Lys  Leu Gly Glu Asp Val  Pro Val Tyr Ile Tyr  Ala Thr Glu
    2315                 2320                 2325

Asp Glu  Asp Leu Ala Val Asp  Leu Leu Gly Leu Asp  Trp Pro Asp
    2330                 2335                 2340

Pro Gly  Asn Gln Gln Val Val  Glu Thr Gly Lys Ala  Leu Lys Gln
    2345                 2350                 2355

Val Thr  Gly Leu Ser Ser Ala  Glu Asn Ala Leu Leu  Val Ala Leu
    2360                 2365                 2370

Phe Gly  Tyr Val Gly Tyr Gln  Ala Leu Ser Lys Arg  His Val Pro
    2375                 2380                 2385

Met Ile  Thr Asp Ile Tyr Thr  Ile Glu Asp Gln Arg  Leu Glu Asp
    2390                 2395                 2400

Thr Thr  His Leu Gln Tyr Ala  Pro Asn Ala Ile Lys  Thr Asp Gly
    2405                 2410                 2415

Thr Glu  Thr Glu Leu Lys Glu  Leu Ala Ser Gly Asp  Val Glu Lys
    2420                 2425                 2430

Ile Met  Gly Ala Ile Ser Asp  Tyr Ala Ala Gly Gly  Leu Glu Phe
    2435                 2440                 2445

Val Lys  Ser Gln Ala Glu Lys  Ile Lys Thr Ala Pro  Leu Phe Lys
    2450                 2455                 2460

Glu Asn  Ala Glu Ala Ala Lys  Gly Tyr Val Gln Lys  Phe Ile Asp
    2465                 2470                 2475

Ser Leu  Ile Glu Asn Lys Glu  Glu Ile Ile Arg Tyr  Gly Leu Trp
    2480                 2485                 2490
```

```
Gly Thr His Thr Ala Leu Tyr Lys Ser Ile Ala Ala Arg Leu Gly
    2495                2500                2505

His Glu Thr Ala Phe Ala Thr Leu Val Leu Lys Trp Leu Ala Phe
    2510                2515                2520

Gly Gly Glu Ser Val Ser Asp His Val Lys Gln Ala Ala Val Asp
    2525                2530                2535

Leu Val Val Tyr Tyr Val Met Asn Lys Pro Ser Phe Pro Gly Asp
    2540                2545                2550

Ser Glu Thr Gln Gln Glu Gly Arg Arg Phe Val Ala Ser Leu Phe
    2555                2560                2565

Ile Ser Ala Leu Ala Thr Tyr Thr Tyr Lys Thr Trp Asn Tyr His
    2570                2575                2580

Asn Leu Ser Lys Val Val Glu Pro Ala Leu Ala Tyr Leu Pro Tyr
    2585                2590                2595

Ala Thr Ser Ala Leu Lys Met Phe Thr Pro Thr Arg Leu Glu Ser
    2600                2605                2610

Val Val Ile Leu Ser Thr Thr Ile Tyr Lys Thr Tyr Leu Ser Ile
    2615                2620                2625

Arg Lys Gly Lys Ser Asp Gly Leu Leu Gly Thr Gly Ile Ser Ala
    2630                2635                2640

Ala Met Glu Ile Leu Ser Gln Asn Pro Val Ser Val Gly Ile Ser
    2645                2650                2655

Val Met Leu Gly Val Gly Ala Ile Ala Ala His Asn Ala Ile Glu
    2660                2665                2670

Ser Ser Glu Gln Lys Arg Thr Leu Leu Met Lys Val Phe Val Lys
    2675                2680                2685

Asn Phe Leu Asp Gln Ala Ala Thr Asp Glu Leu Val Lys Glu Asn
    2690                2695                2700

Pro Glu Lys Ile Ile Met Ala Leu Phe Glu Ala Val Gln Thr Ile
    2705                2710                2715

Gly Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr
    2720                2725                2730
```

Lys Gly Trp Glu Ala Lys Glu Leu Ser Glu Arg Thr Ala Gly Arg
2735 2740 2745

Asn Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly
2750 2755 2760

Met Asp Ser Gln Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile
2765 2770 2775

Leu Asp Leu Ile Tyr Gly Leu His Lys Gln Ile Asn Arg Gly Leu
2780 2785 2790

Lys Lys Met Val Leu Gly Trp Ala Pro Ala Pro Phe Ser Cys Asp
2795 2800 2805

Trp Thr Pro Ser Asp Glu Arg Ile Arg Leu Pro Thr Asp Asn Tyr
2810 2815 2820

Leu Arg Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala
2825 2830 2835

Phe Lys Asn Val Gly Gly Lys Leu Thr Lys Val Glu Glu Ser Gly
2840 2845 2850

Pro Phe Leu Cys Arg Asn Arg Pro Gly Arg Gly Pro Val Asn Tyr
2855 2860 2865

Arg Val Thr Lys Tyr Tyr Asp Asp Asn Leu Arg Glu Ile Lys Pro
2870 2875 2880

Val Ala Lys Leu Glu Gly Gln Val Glu His Tyr Tyr Lys Gly Val
2885 2890 2895

Thr Ala Lys Ile Asp Tyr Ser Lys Gly Lys Met Leu Leu Ala Thr
2900 2905 2910

Asp Lys Trp Glu Val Glu His Gly Val Ile Thr Arg Leu Ala Lys
2915 2920 2925

Arg Tyr Thr Gly Val Gly Phe Asn Gly Ala Tyr Leu Gly Asp Glu
2930 2935 2940

Pro Asn His Arg Ala Leu Val Glu Arg Asp Cys Ala Thr Ile Thr
2945 2950 2955

Lys Asn Thr Val Gln Phe Leu Lys Met Lys Lys Gly Cys Ala Phe
2960 2965 2970

```
Thr Tyr Asp Leu Thr Ile Ser Asn Leu Thr Arg Leu Ile Glu Leu
    2975                2980                2985

Val His Arg Asn Asn Leu Glu Glu Lys Glu Ile Pro Thr Ala Thr
    2990                2995                3000

Val Thr Thr Trp Leu Ala Tyr Thr Phe Val Asn Glu Asp Val Gly
    3005                3010                3015

Thr Ile Lys Pro Val Leu Gly Glu Arg Val Ile Pro Asp Pro Val
    3020                3025                3030

Val Asp Ile Asn Leu Gln Pro Glu Val Gln Val Asp Thr Ser Glu
    3035                3040                3045

Val Gly Ile Thr Ile Ile Gly Arg Glu Thr Leu Met Thr Thr Gly
    3050                3055                3060

Val Thr Pro Val Leu Glu Lys Val Glu Pro Asp Ala Ser Asp Asn
    3065                3070                3075

Gln Asn Ser Val Lys Ile Gly Leu Asp Glu Gly Asn Tyr Pro Gly
    3080                3085                3090

Pro Gly Ile Gln Thr His Thr Leu Thr Glu Glu Ile His Asn Arg
    3095                3100                3105

Asp Ala Arg Pro Phe Ile Met Ile Leu Gly Ser Arg Asn Ser Ile
    3110                3115                3120

Ser Asn Arg Ala Lys Thr Ala Arg Asn Ile Asn Leu Tyr Thr Gly
    3125                3130                3135

Asn Asp Pro Arg Glu Ile Arg Asp Leu Met Ala Ala Gly Arg Met
    3140                3145                3150

Leu Val Val Ala Leu Arg Asp Val Asp Pro Glu Leu Ser Glu Met
    3155                3160                3165

Val Asp Phe Lys Gly Thr Phe Leu Asp Arg Glu Ala Leu Glu Ala
    3170                3175                3180

Leu Ser Leu Gly Gln Pro Lys Pro Lys Gln Val Thr Lys Glu Ala
    3185                3190                3195

Val Arg Asn Leu Ile Glu Gln Lys Lys Asp Val Glu Ile Pro Asn
    3200                3205                3210
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Trp Phe Ala Ser Asp Asp Pro Val Phe Leu Glu Val Ala Leu Lys
    3215                3220                3225

Asn Asp Lys Tyr Tyr Leu Val Gly Asp Val Gly Glu Leu Lys Asp
    3230                3235                3240

Gln Ala Lys Ala Leu Gly Ala Thr Asp Gln Thr Arg Ile Ile Lys
    3245                3250                3255

Glu Val Gly Ser Arg Thr Tyr Ala Met Lys Leu Ser Ser Trp Phe
    3260                3265                3270

Leu Lys Ala Ser Asn Lys Gln Met Ser Leu Thr Pro Leu Phe Glu
    3275                3280                3285

Glu Leu Leu Leu Arg Cys Pro Pro Ala Thr Lys Ser Asn Lys Gly
    3290                3295                3300

His Met Ala Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Glu Pro
    3305                3310                3315

Leu Gly Cys Gly Val His Leu Gly Thr Ile Pro Ala Arg Arg Val
    3320                3325                3330

Lys Ile His Pro Tyr Glu Ala Tyr Leu Lys Leu Lys Asp Phe Ile
    3335                3340                3345

Glu Glu Glu Glu Lys Lys Pro Arg Val Lys Asp Thr Val Ile Arg
    3350                3355                3360

Glu His Asn Lys Trp Ile Leu Lys Lys Ile Arg Phe Gln Gly Asn
    3365                3370                3375

Leu Asn Thr Lys Lys Met Leu Asn Pro Gly Lys Leu Ser Glu Gln
    3380                3385                3390

Leu Asp Arg Glu Gly Arg Lys Arg Asn Ile Tyr Asn His Gln Ile
    3395                3400                3405

Gly Thr Ile Met Ser Ser Ala Gly Ile Arg Leu Glu Lys Leu Pro
    3410                3415                3420

Ile Val Arg Ala Gln Thr Asp Thr Lys Thr Phe His Glu Ala Ile
    3425                3430                3435

Arg Asp Lys Ile Asp Lys Ser Glu Asn Arg Gln Asn Pro Glu Leu
    3440                3445                3450
```

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His Asn  Lys Leu Leu Glu Ile  Phe His Thr Ile Ala  Gln Pro Thr
    3455                 3460                 3465

Leu Lys  His Thr Tyr Gly Glu  Val Thr Trp Glu Gln  Leu Glu Ala
    3470                 3475                 3480

Gly Val  Asn Arg Lys Gly Ala  Ala Gly Phe Leu Glu  Lys Lys Asn
    3485                 3490                 3495

Ile Gly  Glu Val Leu Asp Ser  Glu Lys His Leu Val  Glu Gln Leu
    3500                 3505                 3510

Val Arg  Asp Leu Lys Ala Gly  Arg Lys Ile Lys Tyr  Tyr Glu Thr
    3515                 3520                 3525

Ala Ile  Pro Lys Asn Glu Lys  Arg Asp Val Ser Asp  Asp Trp Gln
    3530                 3535                 3540

Ala Gly  Asp Leu Val Val Glu  Lys Arg Pro Arg Val  Ile Gln Tyr
    3545                 3550                 3555

Pro Glu  Ala Lys Thr Arg Leu  Ala Ile Thr Lys Val  Met Tyr Asn
    3560                 3565                 3570

Trp Val  Lys Gln Gln Pro Val  Val Ile Pro Gly Tyr  Glu Gly Lys
    3575                 3580                 3585

Thr Pro  Leu Phe Asn Ile Phe  Asp Lys Val Arg Lys  Glu Trp Asp
    3590                 3595                 3600

Ser Phe  Asn Glu Pro Val Ala  Val Ser Phe Asp Thr  Lys Ala Trp
    3605                 3610                 3615

Asp Thr  Gln Val Thr Ser Lys  Asp Leu Gln Leu Ile  Gly Glu Ile
    3620                 3625                 3630

Gln Lys  Tyr Tyr Tyr Lys Lys  Glu Trp His Lys Phe  Ile Asp Thr
    3635                 3640                 3645

Ile Thr  Asp His Met Thr Glu  Val Pro Val Ile Thr  Ala Asp Gly
    3650                 3655                 3660

Glu Val  Tyr Ile Arg Asn Gly  Gln Arg Gly Ser Gly  Gln Pro Asp
    3665                 3670                 3675

Thr Ser  Ala Gly Asn Ser Met  Leu Asn Val Leu Thr  Met Met Tyr
    3680                 3685                 3690
```

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Gly Phe Cys Glu Ser Thr Gly Val Pro Tyr Lys Ser Phe Asn Arg
    3695                3700                3705

Val Ala Arg Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile Thr
    3710                3715                3720

Glu Lys Gly Leu Gly Leu Lys Phe Ala Asn Lys Gly Met Gln Ile
    3725                3730                3735

Leu His Glu Ala Gly Lys Pro Gln Lys Ile Thr Glu Gly Glu Lys
    3740                3745                3750

Met Lys Val Ala Tyr Arg Phe Glu Asp Ile Glu Phe Cys Ser His
    3755                3760                3765

Thr Pro Val Pro Val Arg Trp Ser Asp Asn Thr Ser Ser His Met
    3770                3775                3780

Ala Gly Arg Asp Thr Ala Val Ile Leu Ser Lys Met Ala Thr Arg
    3785                3790                3795

Leu Asp Ser Ser Gly Glu Arg Gly Thr Thr Ala Tyr Glu Lys Ala
    3800                3805                3810

Val Ala Phe Ser Phe Leu Leu Met Tyr Ser Trp Asn Pro Leu Val
    3815                3820                3825

Arg Arg Ile Cys Leu Leu Val Leu Ser Gln Gln Pro Glu Thr Asp
    3830                3835                3840

Pro Ser Lys His Ala Thr Tyr Tyr Tyr Lys Gly Asp Pro Ile Gly
    3845                3850                3855

Ala Tyr Lys Asp Val Ile Gly Arg Asn Leu Ser Glu Leu Lys Arg
    3860                3865                3870

Thr Gly Phe Glu Lys Leu Ala Asn Leu Asn Leu Ser Leu Ser Thr
    3875                3880                3885

Leu Gly Val Trp Thr Lys His Thr Ser Lys Arg Ile Ile Gln Asp
    3890                3895                3900

Cys Val Ala Ile Gly Lys Glu Glu Gly Asn Trp Leu Val Lys Pro
    3905                3910                3915

Asp Arg Leu Ile Ser Ser Lys Thr Gly His Leu Tyr Ile Pro Asp
    3920                3925                3930
```

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Lys Gly Phe Thr Leu Gln Gly Lys His Tyr Glu Gln Leu Gln Leu
    3935                3940                3945

Arg Thr Glu Thr Asn Pro Val Met Gly Val Gly Thr Glu Arg Tyr
    3950                3955                3960

Lys Leu Gly Pro Ile Val Asn Leu Leu Leu Arg Arg Leu Lys Ile
    3965                3970                3975

Leu Leu Met Thr Ala Val Gly Val Ser Ser
    3980                3985
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<211> 3898
<212> PRT
<213> Bovine Viral Diarrhea Virus 1

<400> 6

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Met Glu Leu Ile Thr Asn Glu Leu Leu Tyr Lys Thr Tyr Lys Gln Lys
1               5                   10                  15

Pro Val Gly Val Glu Glu Pro Val Tyr Asp Gln Ala Gly Asn Pro Leu
            20                  25                  30

Phe Gly Glu Arg Gly Ala Ile His Pro Gln Ser Thr Leu Lys Leu Pro
        35                  40                  45

His Lys Arg Gly Glu Arg Asn Val Pro Thr Ser Leu Ala Ser Leu Pro
    50                  55                  60

Lys Arg Gly Asp Cys Arg Ser Gly Asn Ser Lys Gly Pro Val Ser Gly
65                  70                  75                  80

Ile Tyr Leu Lys Pro Gly Pro Leu Phe Tyr Gln Asp Tyr Lys Gly Pro
                85                  90                  95

Val Tyr His Arg Ala Pro Leu Glu Leu Phe Glu Glu Gly Ser Met Cys
            100                 105                 110

Glu Thr Thr Lys Arg Ile Gly Arg Val Thr Gly Ser Asp Gly Lys Leu
        115                 120                 125

Tyr His Ile Tyr Ile Cys Ile Asp Gly Cys Ile Thr Val Lys Ser Ala
    130                 135                 140

Thr Arg Ser His Gln Arg Val Leu Arg Trp Val His Asn Arg Leu Asp
145                 150                 155                 160

Cys Pro Leu Trp Val Thr Ser Cys Ser Asp Thr Lys Glu Glu Gly Ala
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                165                 170                 175

Thr Lys Lys Lys Gln Gln Lys Pro Asp Arg Leu Glu Lys Gly Arg Met
            180                 185                 190

Lys Ile Val Pro Lys Glu Ser Glu Lys Asp Ser Lys Thr Lys Pro Pro
        195                 200                 205

Asp Ala Thr Ile Val Val Asp Gly Val Lys Tyr Gln Val Lys Lys Lys
    210                 215                 220

Gly Lys Val Lys Ser Lys Asn Thr Gln Asp Gly Leu Tyr His Asn Lys
225                 230                 235                 240

Asn Lys Pro Pro Glu Ser Arg Lys Lys Leu Glu Lys Ala Leu Leu Ala
                245                 250                 255

Trp Ala Ile Leu Ala Val Val Leu Ile Glu Val Thr Met Gly Glu Asn
            260                 265                 270

Ile Thr Gln Trp Asn Leu Gln Asp Asn Gly Thr Glu Gly Ile Gln Arg
        275                 280                 285

Ala Met Phe Gln Arg Gly Val Asn Arg Ser Leu His Gly Ile Trp Pro
    290                 295                 300

Glu Lys Ile Cys Thr Gly Val Pro Ser His Leu Ala Thr Asp Val Glu
305                 310                 315                 320

Leu Lys Thr Ile His Gly Met Met Asp Ala Ser Glu Lys Thr Asn Tyr
                325                 330                 335

Thr Cys Cys Arg Leu Gln Arg His Glu Trp Asn Lys His Gly Trp Cys
            340                 345                 350

Asn Trp Tyr Asn Ile Glu Pro Trp Ile Leu Ile Met Asn Arg Thr Gln
        355                 360                 365

Ala Asn Leu Thr Glu Gly Gln Pro Pro Arg Glu Cys Ala Val Thr Cys
    370                 375                 380

Arg Tyr Asp Arg Asp Ser Asp Leu Asn Val Val Thr Gln Ala Arg Asp
385                 390                 395                 400

Ser Pro Thr Pro Leu Thr Gly Cys Lys Lys Gly Lys Asn Phe Ser Phe
                405                 410                 415

Ala Gly Val Leu Thr Arg Gly Pro Cys Asn Phe Glu Ile Ala Ala Ser
```

```
            420                 425                 430

Asp Val Leu Phe Lys Glu His Glu Cys Thr Gly Val Phe Gln Asp Thr
        435                 440                 445

Ala His Tyr Leu Val Asp Gly Val Thr Asn Ser Leu Glu Ser Ala Arg
    450                 455                 460

Gln Gly Thr Ala Lys Leu Thr Thr Trp Leu Gly Lys Gln Leu Gly Ile
465                 470                 475                 480

Leu Gly Lys Lys Leu Glu Asn Lys Ser Lys Thr Trp Phe Gly Ala Tyr
                485                 490                 495

Ala Ala Ser Pro Tyr Cys Asp Val Asp Arg Lys Ile Gly Tyr Ile Trp
            500                 505                 510

Phe Thr Lys Asn Cys Thr Pro Ala Cys Leu Pro Lys Asn Thr Lys Ile
        515                 520                 525

Ile Gly Pro Gly Lys Phe Asp Thr Asn Ala Glu Asp Gly Lys Ile Leu
    530                 535                 540

His Glu Met Gly Gly His Leu Ser Glu Val Leu Leu Leu Ser Leu Val
545                 550                 555                 560

Val Leu Ser Asp Phe Ala Pro Glu Thr Ala Ser Ala Met Tyr Leu Ile
                565                 570                 575

Leu His Phe Ser Ile Pro Gln Ser His Val Asp Ile Thr Asp Cys Asp
            580                 585                 590

Lys Thr Gln Leu Asn Leu Thr Ile Glu Leu Thr Thr Ala Asp Val Ile
        595                 600                 605

Pro Gly Ser Val Trp Asn Leu Gly Lys Tyr Val Cys Ile Arg Pro Asp
    610                 615                 620

Trp Trp Pro Tyr Glu Thr Ala Ala Val Leu Ala Phe Glu Glu Val Gly
625                 630                 635                 640

Gln Val Val Lys Ile Val Leu Arg Ala Leu Arg Asp Leu Thr Arg Ile
                645                 650                 655

Trp Asn Ala Ala Thr Thr Thr Ala Phe Leu Val Cys Leu Ile Lys Met
            660                 665                 670

Val Arg Gly Gln Val Val Gln Gly Ile Leu Trp Leu Leu Leu Ile Thr
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        675                 680                 685

Gly Val Gln Gly His Leu Asp Cys Lys Pro Glu Tyr Ser Tyr Ala Ile
    690                 695                 700

Ala Lys Asn Asp Arg Val Gly Pro Leu Gly Ala Glu Gly Leu Thr Thr
705                 710                 715                 720

Val Trp Lys Asp Tyr Ser His Glu Met Lys Leu Glu Asp Thr Met Val
                725                 730                 735

Ile Ala Trp Cys Lys Gly Gly Lys Phe Thr Tyr Leu Ser Arg Cys Thr
            740                 745                 750

Arg Glu Thr Arg Tyr Leu Ala Ile Leu His Ser Arg Ala Leu Pro Thr
        755                 760                 765

Ser Val Val Phe Lys Lys Leu Phe Glu Gly Gln Lys Gln Glu Asp Thr
    770                 775                 780

Val Glu Met Asp Asp Asp Phe Glu Phe Gly Leu Cys Pro Cys Asp Ala
785                 790                 795                 800

Lys Pro Ile Val Arg Gly Lys Phe Asn Thr Thr Leu Leu Asn Gly Pro
                805                 810                 815

Ala Phe Gln Met Val Cys Pro Ile Gly Trp Thr Gly Thr Val Ser Cys
            820                 825                 830

Met Leu Ala Asn Arg Asp Thr Leu Asp Thr Ala Val Val Arg Thr Tyr
        835                 840                 845

Arg Arg Ser Val Pro Phe Pro Tyr Arg Gln Gly Cys Ile Thr Gln Lys
    850                 855                 860

Thr Leu Gly Glu Asp Leu Tyr Asp Cys Ala Leu Gly Gly Asn Trp Thr
865                 870                 875                 880

Cys Val Thr Gly Asp Gln Ser Arg Tyr Thr Gly Gly Leu Ile Glu Ser
                885                 890                 895

Cys Lys Trp Cys Gly Tyr Lys Phe Gln Lys Ser Glu Gly Leu Pro His
            900                 905                 910

Tyr Pro Ile Gly Lys Cys Arg Leu Asn Asn Glu Thr Gly Tyr Arg Leu
        915                 920                 925

Val Asp Asp Thr Ser Cys Asp Arg Glu Gly Val Ala Ile Val Pro His
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    930                 935                 940

Gly Leu Val Lys Cys Lys Ile Gly Asp Thr Thr Val Gln Val Ile Ala
945                 950                 955                 960

Thr Asp Thr Lys Leu Gly Pro Met Pro Cys Lys Pro His Glu Ile Ile
                965                 970                 975

Ser Ser Glu Gly Pro Ile Glu Lys Thr Ala Cys Thr Phe Asn Tyr Thr
            980                 985                 990

Arg Thr Leu Lys Asn Lys Tyr Phe Glu Pro Arg Asp Ser Tyr Phe Gln
        995                 1000                1005

Gln Tyr Met Leu Lys Gly Asp Tyr Gln Tyr Trp Phe Asp Leu Glu
    1010                1015                1020

Val Thr Asp His His Arg Asp Tyr Phe Ala Glu Ser Ile Leu Val
    1025                1030                1035

Val Val Val Ala Leu Leu Gly Gly Arg Tyr Val Leu Trp Leu Leu
    1040                1045                1050

Val Thr Tyr Met Val Leu Ser Glu Gln Lys Ala Ser Gly Ala Gln
    1055                1060                1065

Tyr Gly Ala Gly Glu Val Val Met Met Gly Asn Leu Leu Thr His
    1070                1075                1080

Asp Asn Val Glu Val Val Thr Tyr Phe Phe Leu Leu Tyr Leu Leu
    1085                1090                1095

Leu Arg Glu Glu Ser Val Lys Lys Trp Val Leu Leu Leu Tyr His
    1100                1105                1110

Ile Leu Val Ala His Pro Leu Lys Ser Val Ile Val Ile Leu Leu
    1115                1120                1125

Met Ile Gly Asp Val Val Lys Ala Asp Pro Gly Gly Gln Gly Tyr
    1130                1135                1140

Leu Gly Gln Ile Asp Val Cys Phe Thr Met Val Val Ile Ile Ile
    1145                1150                1155

Ile Gly Leu Ile Ile Ala Arg Arg Asp Pro Thr Ile Val Pro Leu
    1160                1165                1170

Ile Thr Ile Val Ala Ser Leu Arg Val Thr Gly Leu Thr Tyr Ser
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          1175                1180                1185

Pro Gly  Val Asp Ala Ala Met  Ala Val Ile Thr Ile  Thr Leu Leu
    1190                1195                1200

Met Val  Ser Tyr Val Thr Asp  Tyr Phe Arg Tyr Lys  Arg Trp Leu
    1205                1210                1215

Gln Cys  Ile Leu Ser Leu Val  Ser Gly Val Phe Leu  Ile Arg Cys
    1220                1225                1230

Leu Ile  His Leu Gly Arg Ile  Glu Thr Pro Glu Val  Thr Ile Pro
    1235                1240                1245

Asn Trp  Arg Pro Leu Thr Leu  Ile Leu Phe Tyr Leu  Ile Ser Thr
    1250                1255                1260

Thr Val  Val Thr Met Trp Lys  Ile Asp Leu Ala Gly  Leu Leu Leu
    1265                1270                1275

Gln Gly  Val Pro Ile Leu Leu  Leu Ile Thr Thr Leu  Trp Ala Asp
    1280                1285                1290

Phe Leu  Thr Leu Ile Leu Ile  Leu Pro Thr Tyr Glu  Leu Val Lys
    1295                1300                1305

Leu Tyr  Tyr Leu Lys Thr Ile  Lys Thr Asp Ile Glu  Lys Ser Trp
    1310                1315                1320

Leu Gly  Gly Leu Asp Tyr Lys  Arg Val Asp Ser Ile  Tyr Asp Val
    1325                1330                1335

Asp Glu  Ser Gly Glu Gly Val  Tyr Leu Phe Pro Ser  Arg Gln Lys
    1340                1345                1350

Ala Gln  Lys Asn Phe Ser Met  Leu Leu Pro Leu Val  Arg Ala Thr
    1355                1360                1365

Leu Ile  Ser Cys Val Ser Ser  Lys Trp Gln Leu Ile  Tyr Met Ala
    1370                1375                1380

Tyr Leu  Ser Val Asp Phe Met  Tyr Tyr Met His Arg  Lys Val Ile
    1385                1390                1395

Glu Glu  Ile Ser Gly Gly Thr  Asn Met Ile Ser Arg  Ile Val Ala
    1400                1405                1410

Ala Leu  Ile Glu Leu Asn Trp  Ser Met Glu Glu Glu  Glu Ser Lys
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    1415                1420                  1425

Gly Leu  Lys Lys Phe Tyr Leu  Leu Ser Gly Arg Leu  Arg Asn Leu
    1430                1435                  1440

Ile Ile  Lys His Lys Val Arg  Asn Glu Thr Val Ala  Gly Trp Tyr
    1445                1450                  1455

Gly Glu  Glu Glu Val Tyr Gly  Met Pro Lys Ile Met  Thr Ile Ile
    1460                1465                  1470

Lys Ala  Ser Thr Leu Asn Lys  Asn Lys His Cys Ile  Ile Cys Thr
    1475                1480                  1485

Val Cys  Glu Gly Arg Lys Trp  Lys Gly Gly Thr Cys  Pro Lys Cys
    1490                1495                  1500

Gly Arg  His Gly Lys Pro Ile  Thr Cys Gly Met Ser  Leu Ala Asp
    1505                1510                  1515

Phe Glu  Glu Arg His Tyr Lys  Arg Ile Phe Ile Arg  Glu Gly Asn
    1520                1525                  1530

Phe Glu  Gly Pro Phe Arg Gln  Glu Tyr Asn Gly Phe  Ile Gln Tyr
    1535                1540                  1545

Thr Ala  Arg Gly Gln Leu Phe  Leu Arg Asn Leu Pro  Ile Leu Ala
    1550                1555                  1560

Thr Lys  Val Lys Met Leu Met  Val Gly Asn Leu Gly  Glu Glu Val
    1565                1570                  1575

Gly Asp  Leu Glu His Leu Gly  Trp Ile Leu Arg Gly  Pro Ala Val
    1580                1585                  1590

Cys Lys  Lys Ile Thr Glu His  Glu Arg Cys His Ile  Asn Ile Leu
    1595                1600                  1605

Asp Lys  Leu Thr Ala Phe Phe  Gly Ile Met Pro Arg  Gly Thr Thr
    1610                1615                  1620

Pro Arg  Ala Pro Val Arg Phe  Pro Thr Ser Leu Leu  Lys Val Arg
    1625                1630                  1635

Arg Gly  Leu Glu Thr Gly Trp  Ala Tyr Thr His Gln  Gly Gly Ile
    1640                1645                  1650

Ser Ser  Val Asp His Val Thr  Ala Gly Lys Asp Leu  Leu Val Cys
```

1655 1660 1665

Asp Ser Met Gly Arg Thr Arg Val Val Cys Gln Ser Asn Asn Lys
1670 1675 1680

Leu Thr Asp Glu Thr Glu Tyr Gly Val Lys Thr Asp Ser Gly Cys
1685 1690 1695

Pro Asp Gly Ala Arg Cys Tyr Val Leu Asn Pro Glu Ala Val Asn
1700 1705 1710

Ile Ser Gly Ser Lys Gly Ala Val Val His Leu Gln Lys Thr Gly
1715 1720 1725

Gly Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe
1730 1735 1740

Asp Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro Ile Phe Glu
1745 1750 1755

Ala Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn
1760 1765 1770

Glu Glu Ser Lys Pro Thr Lys Ile Met Ser Gly Ile Gln Thr Val
1775 1780 1785

Ser Lys Asn Thr Ala Asp Leu Thr Glu Met Val Lys Lys Ile Thr
1790 1795 1800

Ser Met Asn Arg Gly Asp Phe Lys Gln Ile Thr Leu Ala Thr Gly
1805 1810 1815

Ala Gly Lys Thr Thr Glu Leu Pro Lys Ala Val Ile Glu Glu Ile
1820 1825 1830

Gly Arg His Lys Arg Val Leu Val Leu Ile Pro Leu Arg Ala Ala
1835 1840 1845

Ala Glu Ser Val Tyr Gln Tyr Met Arg Leu Lys His Pro Ser Ile
1850 1855 1860

Ser Phe Asn Leu Arg Ile Gly Asp Met Lys Glu Gly Asp Met Ala
1865 1870 1875

Thr Gly Ile Thr Tyr Ala Ser Tyr Gly Tyr Phe Cys Gln Met Pro
1880 1885 1890

Gln Pro Lys Leu Arg Ala Ala Met Val Glu Tyr Ser Tyr Ile Phe

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        1895                      1900                      1905

Leu Asp Glu Tyr His Cys Ala Thr Pro Glu Gln Leu Ala Ile Ile
    1910                      1915                      1920

Gly Lys Ile His Arg Phe Ser Glu Ser Ile Arg Val Val Ala Met
    1925                      1930                      1935

Thr Ala Thr Pro Ala Gly Ser Val Thr Thr Thr Gly Gln Lys His
    1940                      1945                      1950

Pro Ile Glu Glu Phe Ile Ala Pro Glu Val Met Glu Gly Glu Asp
    1955                      1960                      1965

Leu Gly Ser Gln Phe Leu Asp Ile Ala Gly Leu Lys Ile Pro Val
    1970                      1975                      1980

Asp Glu Met Lys Gly Asn Met Leu Val Phe Val Pro Thr Arg Asn
    1985                      1990                      1995

Met Ala Val Glu Val Ala Lys Lys Leu Lys Ala Lys Gly Tyr Asn
    2000                      2005                      2010

Ser Gly Tyr Tyr Tyr Ser Gly Glu Asp Pro Ala Asn Leu Arg Val
    2015                      2020                      2025

Val Thr Ser Gln Ser Pro Tyr Val Ile Val Ala Thr Asn Ala Ile
    2030                      2035                      2040

Glu Ser Gly Val Thr Leu Pro Asp Leu Asp Thr Val Val Asp Thr
    2045                      2050                      2055

Gly Leu Lys Cys Glu Lys Arg Val Arg Val Ser Ser Lys Ile Pro
    2060                      2065                      2070

Phe Ile Val Thr Gly Leu Lys Arg Met Ala Val Thr Val Gly Glu
    2075                      2080                      2085

Gln Ala Gln Arg Arg Gly Arg Val Gly Arg Val Lys Pro Gly Arg
    2090                      2095                      2100

Tyr Tyr Arg Ser Gln Glu Thr Ala Thr Gly Ser Lys Asp Tyr His
    2105                      2110                      2115

Tyr Asp Leu Leu Gln Ala Gln Arg Tyr Gly Ile Glu Asp Gly Ile
    2120                      2125                      2130

Asn Val Thr Lys Ser Phe Arg Glu Met Asn Tyr Asp Trp Ser Leu
```

2135 2140 2145

Tyr Glu Glu Asp Ser Leu Leu Ile Thr Gln Leu Glu Ile Leu Asn
2150 2155 2160

Asn Leu Leu Ile Ser Glu Asp Leu Pro Ala Ala Val Lys Asn Ile
2165 2170 2175

Met Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala Tyr Asn
2180 2185 2190

Ser Tyr Glu Val Gln Val Pro Val Leu Phe Pro Lys Ile Arg Asn
2195 2200 2205

Gly Glu Val Thr Asp Thr Tyr Glu Asn Tyr Ser Phe Leu Asn Ala
2210 2215 2220

Arg Lys Leu Gly Glu Asp Val Pro Val Tyr Ile Tyr Ala Thr Glu
2225 2230 2235

Asp Glu Asp Leu Ala Val Asp Leu Leu Gly Leu Asp Trp Pro Asp
2240 2245 2250

Pro Gly Asn Gln Gln Val Val Glu Thr Gly Lys Ala Leu Lys Gln
2255 2260 2265

Val Ala Gly Leu Ser Ser Ala Glu Asn Ala Leu Leu Val Ala Leu
2270 2275 2280

Phe Gly Tyr Val Gly Tyr Gln Ala Leu Ser Lys Arg His Val Pro
2285 2290 2295

Met Ile Thr Asp Ile Tyr Thr Ile Glu Asp Gln Arg Leu Glu Asp
2300 2305 2310

Thr Thr His Leu Gln Tyr Ala Pro Asn Ala Ile Lys Thr Glu Gly
2315 2320 2325

Thr Glu Thr Glu Leu Lys Glu Leu Ala Ser Gly Asp Val Glu Lys
2330 2335 2340

Ile Met Gly Ala Ile Ser Asp Tyr Ala Ala Gly Gly Leu Asp Phe
2345 2350 2355

Val Lys Ser Gln Ala Glu Lys Ile Lys Thr Ala Pro Leu Phe Lys
2360 2365 2370

Glu Asn Val Glu Ala Ala Arg Gly Tyr Val Gln Lys Leu Ile Asp

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        2375                    2380                    2385

Ser Leu  Ile Glu Asp Lys Asp  Val Ile Ile Arg Tyr  Gly Leu Trp
    2390                 2395                 2400

Gly Thr  His Thr Ala Leu Tyr  Lys Ser Ile Ala Ala  Arg Leu Gly
    2405                 2410                 2415

His Glu  Thr Ala Phe Ala Thr  Leu Val Leu Lys Trp  Leu Ala Phe
    2420                 2425                 2430

Gly Gly  Glu Thr Val Ser Asp  His Ile Arg Gln Ala  Ala Val Asp
    2435                 2440                 2445

Leu Val  Val Tyr Tyr Val Met  Asn Lys Pro Ser Phe  Pro Gly Asp
    2450                 2455                 2460

Thr Glu  Thr Gln Gln Glu Gly  Arg Arg Phe Val Ala  Ser Leu Phe
    2465                 2470                 2475

Ile Ser  Ala Leu Ala Thr Tyr  Thr Tyr Lys Thr Trp  Asn Tyr Asn
    2480                 2485                 2490

Asn Leu  Ser Lys Val Val Glu  Pro Ala Leu Ala Tyr  Leu Pro Tyr
    2495                 2500                 2505

Ala Thr  Ser Ala Leu Lys Met  Phe Thr Pro Thr Arg  Leu Glu Ser
    2510                 2515                 2520

Val Val  Ile Leu Ser Thr Thr  Ile Tyr Lys Thr Tyr  Leu Ser Ile
    2525                 2530                 2535

Arg Lys  Gly Lys Ser Asp Gly  Leu Leu Gly Thr Gly  Ile Ser Ala
    2540                 2545                 2550

Ala Met  Glu Ile Leu Ser Gln  Asn Pro Val Ser Val  Gly Ile Ser
    2555                 2560                 2565

Val Met  Leu Gly Val Gly Ala  Ile Ala Ala His Asn  Ala Ile Glu
    2570                 2575                 2580

Ser Ser  Glu Gln Lys Arg Thr  Leu Leu Met Lys Val  Phe Val Lys
    2585                 2590                 2595

Asn Phe  Leu Asp Gln Ala Ala  Thr Asp Glu Leu Val  Lys Glu Asn
    2600                 2605                 2610

Pro Glu  Lys Ile Ile Met Ala  Leu Phe Glu Ala Val  Gln Thr Ile
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    2615                2620                2625

Gly Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr Gly Val Tyr Tyr
    2630                2635                2640

Lys Gly Trp Glu Ala Lys Glu Leu Ser Glu Arg Thr Ala Gly Arg
    2645                2650                2655

Asn Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu Leu Gly
    2660                2665                2670

Met Asp Ser Glu Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr Ile
    2675                2680                2685

Leu Asp Leu Ile His Gly Leu His Lys Gln Ile Asn Arg Gly Leu
    2690                2695                2700

Lys Lys Ile Val Leu Gly Trp Ala Pro Ala Pro Phe Ser Cys Asp
    2705                2710                2715

Trp Thr Pro Ser Asp Glu Arg Ile Arg Leu Pro Thr Asp Ser Tyr
    2720                2725                2730

Leu Arg Val Glu Thr Lys Cys Pro Cys Gly Tyr Glu Met Lys Ala
    2735                2740                2745

Leu Lys Asn Val Ser Gly Lys Leu Thr Lys Val Glu Glu Ser Gly
    2750                2755                2760

Pro Phe Leu Cys Arg Asn Arg Pro Gly Arg Gly Pro Val Asn Tyr
    2765                2770                2775

Arg Val Thr Lys Tyr Tyr Asp Asp Asn Leu Arg Glu Ile Arg Pro
    2780                2785                2790

Val Ala Lys Leu Glu Gly Gln Val Glu His Tyr Tyr Lys Gly Val
    2795                2800                2805

Thr Ala Arg Ile Asp Tyr Ser Lys Gly Lys Thr Leu Leu Ala Thr
    2810                2815                2820

Asp Lys Trp Glu Val Glu His Gly Thr Leu Thr Arg Leu Thr Lys
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Arg Tyr Thr Gly Val Gly Phe Arg Gly Ala Tyr Leu Gly Asp Glu
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Pro Asn His Arg Asp Leu Val Glu Arg Asp Cys Ala Thr Ile Thr
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        2855                    2860                    2865

    Lys Asn  Thr Val Gln Phe Leu  Lys Met Lys Lys Gly  Cys Ala Phe
        2870                    2875                    2880

    Thr Tyr  Asp Leu Thr Ile Ser  Asn Leu Thr Arg Leu  Ile Glu Leu
        2885                    2890                    2895

    Val His  Arg Asn Asn Leu Glu  Glu Lys Glu Ile Pro  Thr Ala Thr
        2900                    2905                    2910

    Val Thr  Thr Trp Leu Ala Tyr  Thr Phe Val Asn Glu  Asp Val Gly
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    Thr Ile  Lys Pro Val Leu Gly  Glu Arg Val Ile Pro  Asp Pro Val
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    Val Asp  Ile Asn Leu Gln Pro  Glu Val Gln Val Asp  Thr Ser Glu
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    Val Gly  Ile Thr Ile Ile Gly  Lys Glu Ala Val Met  Thr Thr Gly
        2960                    2965                    2970

    Val Thr  Pro Val Met Glu Lys  Val Glu Pro Asp Thr  Asp Asn Asn
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    Gln Ser  Ser Val Lys Ile Gly  Leu Asp Glu Gly Asn  Tyr Pro Gly
        2990                    2995                    3000

    Pro Gly  Val Gln Thr His Thr  Leu Val Glu Glu Ile  His Asn Lys
        3005                    3010                    3015

    Asp Ala  Arg Pro Phe Ile Met  Val Leu Gly Ser Lys  Ser Ser Met
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    Ser Asn  Arg Ala Lys Thr Ala  Arg Asn Ile Asn Leu  Tyr Thr Gly
        3035                    3040                    3045

    Asn Asp  Pro Arg Glu Ile Arg  Asp Leu Met Ala Glu  Gly Arg Ile
        3050                    3055                    3060

    Leu Val  Val Ala Leu Arg Asp  Ile Asp Pro Asp Leu  Ser Glu Leu
        3065                    3070                    3075

    Val Asp  Phe Lys Gly Thr Phe  Leu Asp Arg Glu Ala  Leu Glu Ala
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    Leu Ser  Leu Gly Gln Pro Lys  Pro Lys Gln Val Thr  Lys Ala Ala
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Lys Asp Lys Tyr His Leu Ile Gly Asp Val Val Glu Val Lys Asp
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Gln Ala Lys Ala Leu Gly Ala Thr Asp Gln Thr Arg Ile Val Lys
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Glu Val Gly Ser Arg Thr Tyr Thr Met Lys Leu Ser Ser Trp Phe
    3170                3175                3180

Leu Gln Ala Ser Ser Lys Gln Met Ser Leu Thr Pro Leu Phe Glu
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Glu Leu Leu Leu Arg Cys Pro Pro Ala Thr Lys Ser Asn Lys Gly
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His Met Ala Ser Ala Tyr Gln Leu Ala Gln Gly Asn Trp Glu Pro
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Leu Gly Cys Gly Val His Leu Gly Thr Val Pro Ala Arg Arg Val
    3230                3235                3240

Lys Met His Pro Tyr Glu Ala Tyr Leu Lys Leu Lys Asp Leu Val
    3245                3250                3255

Glu Glu Glu Glu Lys Lys Pro Arg Ile Arg Asp Thr Val Ile Arg
    3260                3265                3270

Glu His Asn Lys Trp Ile Leu Lys Lys Ile Lys Phe Gln Gly Asn
    3275                3280                3285

Leu Asn Thr Lys Lys Met Leu Asn Pro Gly Lys Leu Ser Glu Gln
    3290                3295                3300

Leu Asp Arg Glu Gly His Lys Arg Asn Ile Tyr Asn Asn Gln Ile
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Ser Thr Val Met Ser Ser Ala Gly Ile Arg Leu Glu Lys Leu Pro
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Leu Lys His Thr Tyr Gly Glu Val Thr Trp Glu Gln Leu Glu Ala
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Gly Ile Asn Arg Lys Gly Ala Ala Gly Phe Leu Glu Lys Lys Asn
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Ala Ile Pro Lys Asn Glu Lys Arg Asp Val Ser Asp Asp Trp Gln
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Ala Gly Asp Leu Val Asp Glu Lys Lys Pro Arg Val Ile Gln Tyr
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Trp Val Lys Gln Gln Pro Val Val Ile Pro Gly Tyr Glu Gly Lys
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Thr Pro Leu Phe Asn Ile Phe Asn Lys Val Arg Lys Glu Trp Asp
    3500                3505                3510

Leu Phe Asn Glu Pro Val Ala Val Ser Phe Asp Thr Lys Ala Trp
    3515                3520                3525

Asp Thr Gln Val Thr Ser Arg Asp Leu His Leu Ile Gly Glu Ile
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Gln Lys Tyr Tyr Tyr Arg Lys Glu Trp His Lys Phe Ile Asp Thr
    3545                3550                3555

Ile Thr Asp His Met Val Glu Val Pro Val Ile Thr Ala Asp Gly
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Glu Val Tyr Ile Arg Asn Gly Gln Arg Gly Ser Gly Gln Pro Asp
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Val Ala Lys Ile His Val Cys Gly Asp Asp Gly Phe Leu Ile Thr
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Glu Lys Gly Leu Gly Leu Lys Phe Ser Asn Lys Gly Met Gln Ile
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Leu His Glu Ala Gly Lys Pro Gln Lys Leu Thr Glu Gly Glu Lys
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Met Lys Val Ala Tyr Lys Phe Glu Asp Ile Glu Phe Cys Ser His
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Pro Ser Thr Gln Thr Thr Tyr Tyr Tyr Lys Gly Asp Pro Ile Gly
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Thr Gly Phe Glu Lys Leu Ala Asn Leu Asn Leu Ser Leu Ser Thr
3785 3790 3795

Leu Gly Ile Trp Thr Lys His Thr Ser Lys Arg Ile Ile Gln Asp
3800 3805 3810

Cys Val Ala Ile Gly Lys Glu Glu Gly Asn Trp Leu Val Asn Ala

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    3830                3835                3840

Lys Gly Phe Thr Leu Gln Gly Lys His Tyr Glu Gln Leu Gln Leu
    3845                3850                3855

Gly Ala Glu Thr Asn Pro Val Met Gly Val Gly Thr Glu Arg Tyr
    3860                3865                3870

Lys Leu Gly Pro Ile Val Asn Leu Leu Leu Arg Arg Leu Lys Val
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Leu Leu Met Ala Ala Val Gly Ala Ser Ser
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Claims

1. A vaccine composition comprising at least two live mutant viruses of the same family, wherein each virus contains a mutation in the viral genome, and the mutations in the viruses reside in the same genomic site such that the mutant viruses cannot recombine with each other to eliminate the mutations, and wherein two of the live mutant viruses consist of mutant Bovine Viral Diarrhea Viruses (BVDV).

2. The vaccine composition of claim 1, wherein the two mutant live viruses consist of a mutant Bovine Viral Diarrhea Virus Type 1 (BVDV-1) and a mutant Bovine Viral Diarrhea Virus Type 2 (BVDV-2).

3. The vaccine composition of claim 2, wherein the two mutant live viruses consist of a cytopathic (cp) BVDV-1 and a cp BVDV-2, and are both attenuated.

4. The vaccine composition of claim 3, wherein the cp BVDV-1 and the cp BVDV-2 both comprise a mutation in the NS2-3 region that results in a cytopathic biotype.

5. The vaccine composition of claim 4, wherein the cp BVDV-1 is BVDV-1 NADL, and the cp BVDV-2 is BVDV-2 53637.

6. The vaccine composition of claim 2, further comprising at least one of bovine herpesvirus-1, bovine respiratory syncytial virus, parainfluenza virus-3, *Campylobacter fetus, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagiae, Leptospira pomona,* or *Mannhemia haemolytica.*

7. The vaccine composition of claim 1, further comprising a veterinarily-acceptable carrier.

8. A method of preparing a safe viral vaccine comprising selecting or constructing two live mutant viruses of the same family, wherein each virus contains a mutation and the mutations in the viruses reside in the same genomic site such that the mutant viruses can not undergo homologous recombination to eliminate the mutations, and wherein the viruses consist of mutant BVDV.

9. The method of claim 8, wherein said mutation is selected from a deletion, an insertion, a substitution, or a combination thereof.

10. The method of claim 8, wherein said mutation confers a phenotype selected from attenuation of virulence, alteration of cellular tropism or biotype, alteration of species tropism, expression of a foreign gene cassette, or a combination thereof.

11. The method of claim 8, wherein the two mutant live viruses consist of a mutant BVDV-1 and a mutant BVDV-2.

Patentansprüche

1. Vakzinzusammensetzung, welche mindestens zwei lebende Virusmutanten der gleichen Familie umfasst, wobei jedes Virus eine Mutation in dem viralen Genom enthält, und die Mutationen in den Viren an der gleichen genomischen Stelle liegen, so dass die Virusmutanten nicht miteinander rekombinieren können, um die Mutationen zu eliminieren, und wobei zwei der lebenden Virusmutanten aus Bovine-Virusdiarrhoe-Virus(BVDV)-Mutanten bestehen.

2. Vakzinzusammensetzung gemäß Anspruch 1, wobei die zwei lebenden Virusmutanten aus einer Bovine-Virusdiarrhoe-Virus-Typ1(BVDV-1)-Mutante und einer Bovine-Virusdiarrhoe-Virus-Typ2(BVDV-2)-Mutante bestehen.

3. Vakzinzusammensetzung gemäß Anspruch 2, wobei die zwei lebenden Virusmutanten aus einem zytopathischen (cp) BVDV-1 und einem cp BVDV-2 bestehen und beide attenuiert sind.

4. Vakzinzusammensetzung gemäß Anspruch 3, wobei beide, das cp BVDV-1 und das cp BVDV-2, eine Mutation in der NS2-3 Region umfassen, welche zu einem zytopathischen Biotyp führt.

5. Vakzinzusammensetzung gemäß Anspruch 4, wobei das cp BVDV-1 BVDV-1 NADL ist und das cp BVDV-2 BVDV-2 53637 ist.

6. Vakzinzusammensetzung gemäß Anspruch 2, welche weiter mindestens eines/eine von Bovines Herpesvirus-1, bovines respiratorisches-Synzytial-Virus, ParainfluenzaVirus-3, *Campylobacter fetus, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icteronaemorrhagiae, Leptospira pomona oder Mannhemia haemolytica* enthält.

7. Vakzinzusammensetzung gemäß Anspruch 1, welche weiter einen verterinärmedizinisch annehmbaren Träger enthält.

8. Verfahren zur Herstellung eines sicheren viralen Vakzins, welches das Auswählen oder Erstellen von zwei lebenden Virusmutanten der gleichen Familie umfasst, wobei jedes Virus eine Mutation enthält und die Mutationen in den Viren an der gleichen genomischen Stelle liegen, so dass die Virusmutanten keine homologe Rekombination durchmachen können, um die Mutationen zu eliminieren, und wobei die Viren aus BVDV-Mutanten bestehen.

9. Verfahren gemäß Anspruch 8, wobei die Mutation aus einer Deletion, einer Insertion, einer Substitution oder einer Kombination davon ausgewählt ist.

10. Verfahren gemäß Anspruch 8, wobei die Mutation einen Phänotyp, ausgewählt aus einer Attenuation der Virulenz, einer Änderung des zellulären Tropismus oder Biotyps, einer Änderung des Speziestropismus, einer Expression von einer fremden Genkassette oder einer Kombination davon, verleiht.

11. Verfahren gemäß Anspruch 8, wobei die zwei lebenden Virusmutanten aus einer BVDV-1-Mutante und einer BVDV-2-Mutante bestehen.

Revendications

1. Composition de vaccin comprenant au moins deux virus mutants vivants de la même famille, dans laquelle chaque virus contient une mutation dans le génome viral, et les mutations dans les virus se trouvent dans le même site génomique de telle sorte que les virus mutants ne puissent pas se recombiner mutuellement pour éliminer les mutations, et dans laquelle deux des virus mutants vivants consistent en virus mutants de la diarrhée virale bovine (BVDV).

2. Composition de vaccin suivant la revendication 1, dans laquelle les deux virus vivants mutants consistent en un virus de la diarrhée virale bovine de type 1 (BVDV-1) mutant et un virus de la diarrhée virale bovine de type 2 (BVDV-2) de type 2.

3. Composition de vaccin suivant la revendication 2, dans laquelle les deux virus vivants mutants consistent en un BVDV-1 cytopathogène (cp) et un BVDV-2 cp et sont tous deux atténués.

4. Composition de vaccin suivant la revendication 3, dans laquelle le BVDV-1 cp et le BVDV-2 cp comprennent tous deux une mutation dans la région NS2-3 ayant pour résultat un biotype cytopathogène.

5. Composition de vaccin suivant la revendication 4, dans laquelle le BVDV-1 cp est le BVDV-1 NADL et le BVDV-2 cp est BVDV-2 53637.

6. Composition de vaccin suivant la revendication 2, comprenant en outre au moins un des herpesvirus-1 bovin, virus syncytial respiratoire bovin, virus parainfluenza3, *Campylobacter fetus, Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagiae, Leptospira pomona* ou *Mannhemia haemolytica.*

7. Composition de vaccin suivant la revendication 1, comprenant en outre un support acceptable du point de vue vétérinaire.

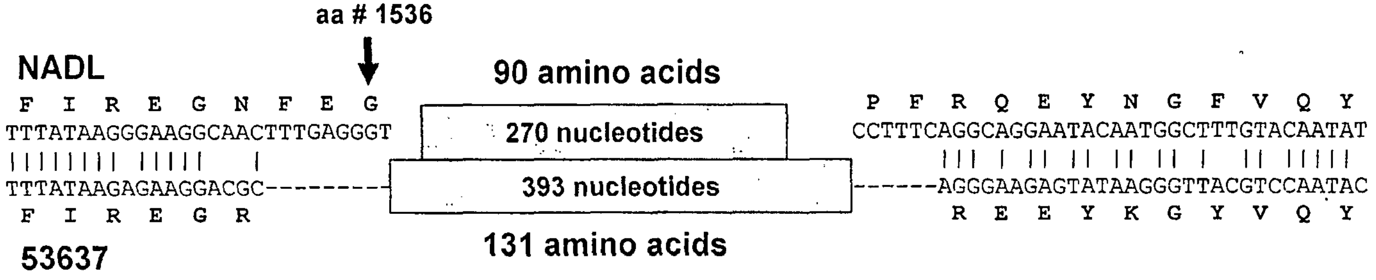
8. Procédé pour la préparation d'un vaccin viral sûr, comprenant la sélection ou la construction de deux virus mutants vivants de la même famille, dans lequel chaque virus contient une mutation et les mutations dans les virus résident dans le même site génomique de telle sorte que les virus mutants ne puissent pas subir de recombinaison homologue pour éliminer les mutations, et dans lequel les virus consistent en BVDV mutant.

9. Procédé suivant la revendication 8, dans lequel ladite mutation est choisie entre une délétion, une insertion, une substitution et leurs combinaisons.

10. Procédé suivant la revendication 8, dans lequel ladite mutation confère un phénotype choisi entre l'atténuation de la virulence, la modification du tropisme cellulaire ou du biotype, la modification du tropisme spécifique, l'expression d'une cassette de gène étrangère et une de leurs combinaisons.

11. Procédé suivant la revendication 8, dans lequel les deux virus vivants mutants consistent en un BVDV-1 mutant et un BVDV-2 mutant.

Figure 1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NADL
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P F R Q E Y N G F V Q Y
CCTTTCAGGCAGGAATACAATGGCTTTGTACAATAT
TTTATAAGAGAAGGACGC
393 nucleotides
AGGGAAGAGTATAAGGGTTACGTCCAATAC
F I R E G R
R E E Y K G Y V Q Y
53637
131 amino acids

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- WO 9835562 A **[0038]**
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