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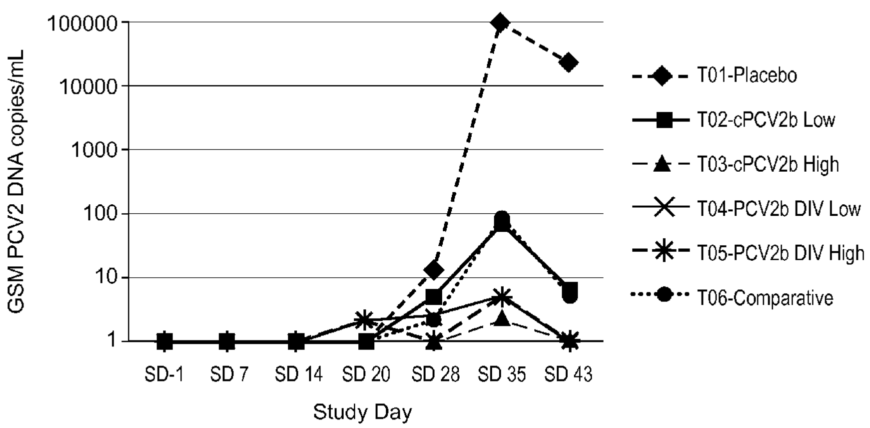
(54) **Title**: PCV2B DIVERGENT VACCINE COMPOSITION AND METHODS OF USE

FIG. 5



(57) **Abstract**: This invention provides a vaccine composition for protecting pigs against PCV2, including a highly virulent porcine circovirus type 2b (PCV2b) divergent strain, the composition including a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide comprises Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1 herein.

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PCV2B DIVERGENT VACCINE COMPOSITION AND METHODS OF USE

Field of the Invention

The present invention relates to porcine circovirus. More particularly, the invention relates to a vaccine composition including a PCV2b divergent ORF2 antigen and its use in a vaccine for protecting pigs against PCV2, including a highly virulent porcine circovirus type 2b (PCV2b) divergent strain, and Post-weaning Multisystemic Wasting Syndrome (PMWS).

Background of the Invention

Porcine circovirus type 2 (PCV2), a member of *Circoviridae* family, genus *Circovirus*, is a small nonenveloped circular virus which was initially discovered in 1998. PCV2 is one of the two most prevalent pathogens encountered in the pig industry, the other being *Mycoplasma hyopneumoniae* (M. hyo). Swine infected with PCV2 exhibit a syndrome commonly referred to as Post-weaning Multisystemic Wasting Syndrome (PMWS). PMWS is clinically characterized by wasting, paleness of the skin, unthriftiness, respiratory distress, diarrhea, icterus, and jaundice. In addition to PMWS, PCV2 has been associated with several other diseases, including pseudorabies, porcine reproductive and respiratory syndrome (PRRS), enzootic pneumonia, Glasser's disease, streptococcal meningitis, salmonellosis, postweaning colibacillosis, dietetic hepatosis, and suppurative bronchopneumonia. The various clinical manifestations of PCV2 infection in pigs across the age groups has become known as porcine circovirus-associated disease (PCVAD), and are characterized by wasting and growth retardation. PRRS virus, Swine Influenza Virus (SIV), M. hyo, and other bacteria have been implicated as major co-factors in the development of PCVAD. PCVAD has continuously been a threat to the global swine industry, causing high economic losses.

PCV2 isolates are currently further subdivided into three genotypes: PCV2a, PCV2b, and PCV2c. PCV2 contains two major open reading frames (ORFs), which encode a protein associated with replication (ORF1, 945 nt), and the virus capsid (ORF2, 702 nt). PCV2 has undergone significant genetic variation in recent years. A newly emergent PCV2 mutant with an additional lysine (K) at the C-terminus of the ORF2-encoded capsid protein compared with classical PCV2a and PCV2b genotypes was isolated in 2008 from a serum sample from an aborted pig (Guo et al., 2010, Virology Journal 7: 273). In this newly emerging PCV2 mutant, a one-base deletion at position 1039 in the genomic sequence resulted in a mutation of the stop codon (from UAA to AAG) in ORF2, to give an ORF2 gene of 705 nt and a new stop codon (Guo et al., 2011, Virology Journal 8: 291). In addition, Knell et al. have reported previously that mutations could occur in the ORF2 gene, because a deletion (T) was found at position 1042 in the 1767 nt genome of one strain (GenBank no. AY713470), which led to elongation by one amino acid (lysine) in the C terminus of the ORF2-encoded capsid protein (Knell et al., 2005, Veterinary Microbiology 109: 169-177). Olvera et al. have also reported elongation by one lysine residue of the C terminus of the capsid protein due to a mutation in the stop codon of ORF2 (Olvera et al., 2007, Virology 357: 175-185). Additionally, a PCV2 strain termed "JSTZ", with GenBank accession No. JQ413808, was detected and identified in stool samples of a piglet with severe diarrhea in China, and its complete 1767 nt genome was sequenced (Li et al., 2012, Journal of Virology (jvi.asm.org), p. 4716). Phylogenetic analyses based on the genome of PCV2 strain JSTZ and the ORFs of other Chinese PCV2 strains indicated that PCV2 strain JSTZ belonged to a novel genotype in China (Li et al., 2012, *supra*).

Guo et al. assessed the relative virulence of a PCV2 mutant strain termed PCV2b/rBDH or BDH (Gen Bank accession No. HM038017), which had been recovered in 2008 from a sample from an aborted pig with PMWS, and confirmed the greater virulence of the PCV2 mutant strain in piglets than that associated with the classical PCV2a and PCV2b genotypes (Guo et al., 2012, PLoS ONE (plosone.org), Vol. 7, Issue 7, e41463, 1-10). This PCV2 mutant strain demonstrated more severe signs compatible with PMWS, characterized by wasting, coughing, dyspnea, diarrhea, rough hair-coat and depression. Moreover, the pathological lesions and viremia, as well as the viral loads in lymph nodes, tonsils, and spleen, were significantly more severe for piglets challenged with the PCV2 mutant strain compared with those in the groups challenged with classical PCV2a and PCV2b. In addition, a significantly lower average daily weight gain was recorded in the group challenged with the PCV2 mutant strain than in the groups challenged with the prevailing PCV2a and PCV2b genotypes (Guo et al., 2012, *supra*).

Two PCV2 strains, US22625-33 and US22664-35, were recently identified in cases of suspected vaccine failure in PMWS-affected pigs in a production system located in the United States (Xiao et al., 2012, Journal of Virology (jvi.asm.org), Vol. 86, No. 22, p. 12469). The full genome of these two US strains was found to be comprised of 1767 nt, and the size of its ORF2 gene was 705 nt, encoding an ORF2 protein of 234 aa, which was one amino acid longer than that of common PCV2. Phylogenetic analysis with the nucleotide sequences of ORF2 of classical PCV2a and PCV2b strains suggested that both U.S. PCV2 strains US22625-33 and US22664-35 are closely related to PCV2b. Compared with classic PCV2b, a single base deletion within the ORF2 gene resulted in the addition of a single amino acid (lysine) to the C-terminus of the ORF2 protein. Further sequence BLAST and comparison showed that both U.S. PCV2 strains had a high level of identity (99.9%) with the PCV2 strain, BDH, found in China, and reported to be of increased virulence. One silent mutation (1677A→1677T) in ORF1 was found between BDH and the two U.S. mutant PCV2s. According to the new PCV2 genotype definition and nomenclature criteria (Cortey, et al., 2011, Vet. Microbiol. 149:522-523; Segales, et al., 2008, Vet. Rec. 162:867-868), all of these novel mutant PCV2 strains could be classified into genotype PCV2b, based on the phylogenetic analysis of the nucleotide sequence of the ORF2 gene (Xiao et al., 2012, *supra*).

In view of the reported increased virulence of the new PCV2b divergent, as well its presence in cases of suspected vaccine failures in the United States, what is needed is an efficacious vaccine against this new PCV2b divergent. Preferably, this vaccine will be compatible with other porcine antigens, such as M. hyo and PRRS virus.

<u>Summary of the Invention</u>

The present invention provides a vaccine composition for protecting pigs against PCV2, including a highly virulent porcine circovirus type 2b (PCV2b) divergent strain, the composition including a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide comprises Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. In one embodiment, the composition also provides heterologous protection against classical PCV2a and PCV2b strains.

In one embodiment, the composition is in the form of an inactivated, PCV2b divergent whole virus that comprises and/or expresses the PCV2b divergent ORF2 polypeptide.

In another embodiment, the composition is in the form of an inactivated chimeric virus, wherein the chimeric virus comprises an inactivated recombinant porcine circovirus type 1 that comprises and/ or expresses the PCV2b divergent ORF2 polypeptide.

In yet another embodiment, the composition is in the form of an isolated, recombinant PCV2b divergent ORF2 polypeptide. In one embodiment, the isolated, recombinant PCV2b divergent ORF2 polypeptide is expressed from a vector. In another embodiment, the vector is a baculovirus or parapoxvirus. In a further embodiment, the vector is a live or inactivated vector.

In one embodiment, the PCV2b divergent ORF2 polypeptide which includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1, further includes at least one residue selected from the group consisting of: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In another embodiment, the PCV2b divergent ORF2 polypeptide which includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1, further includes a Lysine (K) at residue 59 and a Lysine (K) at residue 234, according to the numbering of SEQ ID NO: 1.

In a further embodiment, the PCV2b divergent ORF2 polypeptide which includes Leucine (L) at position 89, Threonine (T) at position 90, Aspargine (N) at position 134, a Lysine (K) at residue 59 and a Lysine (K) at residue 234, according to the numbering of SEQ ID NO: 1, further includes a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment, the PCV2 divergent ORF2 polypeptide is represented by the amino acid sequence of SEQ ID NO: 1, or a fragment thereof.

In another embodiment, the composition including the PCV2 divergent ORF2 polypeptide further includes at least one additional porcine antigen. In one embodiment, the at least one additional antigen is protective against a disease in pigs caused by a microorganism.

In one embodiment, the microorganism includes a bacterium, virus, or protozoan. In another embodiment, the microorganism is selected from, but is not limited to, the following: porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), *Haemophilus parasuis, Pasteurella multocida, Streptococcum suis, Staphylococcus hyicus, Actinobacilllus pleuropneumoniae, Bordetella bronchiseptica, Salmonella choleraesuis, Salmonella enteritidis, Erysipelothrix rhusiopathiae*, *Mycoplama hyorhinis, Mycoplasma hyosynoviae*, leptospira bacteria, *Lawsonia intracellularis*, swine influenza virus (SIV), *Escherichia coli* antigen, *Brachyspira hyodysenteriae*, porcine respiratory coronavirus, Porcine Epidemic Diarrhea (PED) virus, rotavirus, Torque teno virus (TTV), Porcine Cytomegalovirus, Porcine enteroviruses, Encephalomyocarditis virus, a pathogen causative of Aujesky's Disease, Classical Swine fever (CSF) and a pathogen causative of Swine Transmissable Gastroenteritis, or combinations thereof.

In some embodiments, the composition of the present invention further includes an adjuvant. In one embodiment, the adjuvant is selected from, but is not limited to, an oil-in-water adjuvant, a polymer and water adjuvant, a water-in-oil adjuvant, an aluminum hydroxide adjuvant, a vitamin E adjuvant and combinations thereof. In another embodiment, the composition of the present invention further includes a pharmaceutically acceptable carrier.

The present also provides a method of immunizing a pig against PCV2, including a PCV2b divergent strain, the method including administering to the pig a composition of the present invention, as described above. This composition for administration includes a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. As described above, this PCV2b divergent ORF2 polypeptide can further include at least one residue selected from the following: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment, the composition for administration includes a virus comprising and/or expressing the PCV2b divergent ORF2 polypeptide. In another embodiment, the composition for administration includes an isolated, recombinant PCV2b ORF2 polypeptide.

In one embodiment of the method of the present invention, the composition can be administered intramuscularly, intradermally, transdermally, subcutaneously, intranasally, or orally, or by other routes known to those of skill in the art. In another embodiment, the composition is administered in a single dose. In yet another embodiment, the composition is administered as two doses.

In a further embodiment, the composition is administered to pigs having maternally-derived antibodies against PCV2.

In one embodiment, the composition is administered to pigs at 3 weeks of age or older.

The present invention further provides a kit. This kit includes a bottle comprising a vaccine composition according to the present invention for protecting pigs against a highly virulent porcine circovirus type 2b (PCV2b) divergent strain. This vaccine composition includes a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. As described above, this PCV2b divergent ORF2 polypeptide can further include at least one residue selected from the following: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment of the kit, the vaccine composition is in the form of a virus comprising and/or expressing the PCV2b divergent ORF2 polypeptide. In another embodiment of the kit, the vaccine composition is in the form of an isolated, recombinant PCV2b divergent ORF2 polypeptide.

In one embodiment of the kit, the vaccine composition in the bottle is provided as a ready-to-use liquid composition. In another embodiment of the kit, the vaccine composition in the bottle is provided in a lyophilized form. In a further embodiment, the kit can include a diluent. In yet another embodiment, the kit can further include an instruction manual which contains the information for administration of the vaccine composition.

Brief Description of the Drawings

Figure 1 shows the amino acid sequence alignments between the capsid sequence of the PCV2b divergent strain termed “PCV2B-DIV-MUT”, and those of a classical PCV2A strain (termed ISU-40895) and a classical PCV2b strain (termed NMB).

Figure 2 is a graph showing geometric Least Squares Means of DNA copies by treatment day. *All “0”s were converted to 1 for graphing purposes.

Figure 3 is a graph showing geometric Least Squares Means of Fecal Shed (DNA Copies) by treatment day post challenge.*All "0"s were converted to 1 for graphing purposes.

Figure 4 is a graph showing PCV2 ELISA S/P LS Mean titers by treatment day and treatment.

Figure 5 is a graph of backtransformed geometric Least Squares Means of DNA copies by treatment day.

Figure 6 is a graph of backtransformed geometric Least Squares Means of fecal shed (DNA Copies) by treatment day post challenge.

Figure 7 is a graph showing PCV2 ELISA S/P LS Mean Titers by Study Day

Brief Description of the Sequences

As used herein, the PCV2 isolates represented by SEQ ID NOs: 1 to 57 and 66 are representative examples of PCV2b divergent strains.

SEQ ID NO: 1 is the amino acid sequence corresponding to the full-length capsid of a PCV2b divergent strain termed PCV2B-DIV-MUT herein.

SEQ ID NO: 2 is the nucleotide sequence encoding the full-length capsid of a PCV2b divergent strain termed PCV2B-DIV-MUT herein.

SEQ ID NO: 3 is the amino acid sequence corresponding to the full-length capsid of the PCV2 strain: 798-1, with GenBank Accession number AB462384.

SEQ ID NO: 4 is the nucleotide sequence encoding the full-length capsid of the PCV2 strain: 798-1, with GenBank Accession number AB462384.

SEQ ID NO: 5 is the amino acid sequence corresponding to the full-length capsid of the PCV2 strain: FF, with GenBank Accession number DQ231516.

SEQ ID NO: 6 is the nucleotide sequence encoding the full-length capsid of the PCV2 strain: FF, with GenBank Accession number DQ231516.

SEQ ID NO: 7 is the amino acid sequence corresponding to the full-length capsid of the PCV2 strain: VC 2002-k2, with GenBank Accession number EF990645.

SEQ ID NO: 8 is the nucleotide sequence encoding the full-length capsid of the PCV2 strain: VC 2002-k2, with GenBank Accession number EF990645.
SEQ ID NO: 9 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: GY09, with GenBank Accession number GQ845025.
SEQ ID NO: 10 is the nucleotide sequence encoding the full-length capsid of the PCV2 strain: GY09, with GenBank Accession number GQ845025.
SEQ ID NO: 11 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: XS09, with GenBank Accession number GQ845028.
SEQ ID NO: 12 is the nucleotide sequence encoding the full-length capsid of the PCV2 strain: XS09, with GenBank Accession number GQ845028.
SEQ ID NO: 13 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: SDld01, with GenBank Accession number HM535640.
SEQ ID NO: 14 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: SDld01, with GenBank Accession number HM535640.
SEQ ID NO: 15 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: SDld02, with GenBank Accession number HM755880.
SEQ ID NO: 16 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: SDld02, with GenBank Accession number HM755880.
SEQ ID NO: 17 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: HM01, with GenBank Accession number HM755881.
SEQ ID NO: 18 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: HM01, with GenBank Accession number HM755881.
SEQ ID NO: 19 is the amino acid sequence corresponding to the full-length capsid of the PCV2 strain: NIVS-1, with GenBank Accession number HQ378157.
SEQ ID NO: 20 is the nucleotide sequence encoding the full-length capsid of the PCV2 strain: NIVS-1, with GenBank Accession number HQ378157.
SEQ ID NO: 21 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: C/2010-2*, with GenBank Accession number JF683394.
SEQ ID NO: 22 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: C/2010-2*, with GenBank Accession number JF683394.

SEQ ID NO: 23 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: G/2009-2, with GenBank Accession number JF683408.
SEQ ID NO: 24 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: G/2009-2, with GenBank Accession number JF683408.
SEQ ID NO: 25 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: I/2010, with GenBank Accession number JF927984.
SEQ ID NO: 26 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: I/2010, with GenBank Accession number JF927984.
SEQ ID NO: 27 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: J/2010, with GenBank Accession number JF927985.
SEQ ID NO: 28 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: J/2010, with GenBank Accession number JF927985.
SEQ ID NO: 29 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: K/2010, with GenBank Accession number JF927986.
SEQ ID NO: 30 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: K/2010, with GenBank Accession number JF927986.
SEQ ID NO: 31 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: M/2010, with GenBank Accession number JF927988.
SEQ ID NO: 32 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: M/2010, with GenBank Accession number JF927988.
SEQ ID NO: 33 is the amino acid sequence corresponding to the capsid of the PCV2 isolate: WB/ROM89, with GenBank Accession number JN006445.
SEQ ID NO: 34 is the nucleotide sequence encoding the capsid of the PCV2 isolate: WB/ROM89, with GenBank Accession number JN006445.
SEQ ID NO: 35 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: EU-RO-F4-3, with GenBank Accession number JN382188.
SEQ ID NO: 36 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: EU-RO-F4-3, with GenBank Accession number JN382188.
SEQ ID NO: 37 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: HNing09, with GenBank Accession number JN411096.

SEQ ID NO: 38 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: HNing09, with GenBank Accession number JN411096.
SEQ ID NO: 39 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: YWu09, with GenBank Accession number JN411099.
SEQ ID NO: 40 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: YWu09, with GenBank Accession number JN411099.
SEQ ID NO: 41 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: CH-IVT4, with GenBank Accession number JX984586.
SEQ ID NO: 42 is the nucleotide sequence of the full-length capsid gene of the PCV2 isolate: CH-IVT4, with GenBank Accession number JX984586.
SEQ ID NO: 43 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: CH-IVT6, with GenBank Accession number JX984588.
SEQ ID NO: 44 is the nucleotide sequence of the full-length capsid gene of the PCV2 isolate: CH-IVT6, with GenBank Accession number JX984588.
SEQ ID NO: 45 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: CH-IVT7, with GenBank Accession number JX984589.
SEQ ID NO: 46 is the nucleotide sequence of the full-length capsid gene of the PCV2 isolate: CH-IVT7, with GenBank Accession number JX984589.
SEQ ID NO: 47 is the amino acid sequence corresponding to the full-length capsid of a PCV2 isolate, with GenBank Accession number JX984590.
SEQ ID NO: 48 is the nucleotide sequence of the full-length capsid gene of a PCV2 isolate, with GenBank Accession number JX984590.
SEQ ID NO: 49 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: CH-IVT9, with GenBank Accession number JX984591.
SEQ ID NO: 50 is the nucleotide sequence of the full-length capsid gene of the PCV2 isolate: CH-IVT9, with GenBank Accession number JX984591.
SEQ ID NO: 51 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: CH-IVT10, with GenBank Accession number JX984592.
SEQ ID NO: 52 is the nucleotide sequence of the full-length capsid gene of the PCV2 isolate: CH-IVT10, with GenBank Accession number JX984592.

SEQ ID NO: 53 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: CH-IVT11, with GenBank Accession number JX984593.
SEQ ID NO: 54 is the nucleotide sequence of the full-length capsid gene of the PCV2 isolate: CH-IVT11, with GenBank Accession number JX984593.
SEQ ID NO: 55 is the amino acid sequence corresponding to the full-length capsid of the PCV2 isolate: GDYX, with GenBank Accession number JX519293.
SEQ ID NO: 56 is the nucleotide sequence encoding the full-length capsid of the PCV2 isolate: GDYX, with GenBank Accession number JX519293.
SEQ ID NO: 57 is the complete genome sequence of the PCV2 isolate: GDYX, with GenBank Accession number JX519293.
SEQ ID NO: 58 is the amino acid sequence corresponding to the full-length capsid of a classical PCV2a isolate: ISU-40895, with GenBank Accession number AF264042.
SEQ ID NO: 59 is the nucleotide sequence encoding the full-length capsid of a PCV2a isolate: ISU-40895, with GenBank Accession number AF264042.
SEQ ID NO: 60 is the amino acid sequence corresponding to the full-length capsid of a classical PCV2a isolate: Imp.1010-Stoon, with GenBank Accession number AF055392.
SEQ ID NO: 61 is the nucleotide sequence encoding the full-length capsid of a classical PCV2a isolate: Imp.1010-Stoon, with GenBank Accession number AF055392.
SEQ ID NO: 62 is the amino acid sequence corresponding to the full-length capsid of a classical PCV2b strain: NMB, with GenBank Accession number GU799576.
SEQ ID NO: 63 is the nucleotide sequence encoding the full-length capsid of a classical PCV2b isolate: NMB, with GenBank Accession number GU799576.
SEQ ID NO: 64 is the amino acid sequence corresponding to the full-length capsid of a classical PCV2c strain: DK1980PMWSfree, with GenBank Accession number EU148503.
SEQ ID NO: 65 is the nucleotide sequence encoding the full-length capsid of a classical PCV2c strain: DK1980PMWSfree, with GenBank Accession number EU148503.
SEQ ID NO: 66 is the complete genome sequence of the PCV2 divergent termed "PCV2b-DIV-MUT".

Detailed Description of the Invention

As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a protein antigen” includes a plurality of protein antigens, including mixtures thereof.

As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but do not exclude other elements.

As used herein, the terms “PCV2b divergent strain”, “PCV2b divergent”, “PCV2 mutant”, “novel mutant PCV2”, “mutant PCV2”, and the like refer to a highly virulent PCV2b strain which encodes an ORF2 capsid polypeptide that includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. The encoded PCV2b divergent ORF2 polypeptide can further include at least one residue selected from: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215 according to the numbering of SEQ ID NO: 1.

As used herein, the term “a PCV2b divergent ORF2 polypeptide” is intended to include an virus comprising and/or expressing the PCV2b divergent ORF2 polypeptide, such that the ORF2 polypeptide is a component of the virus itself (e.g., protein coat of the virus). The virus can be PCV, but should not be construed to be limited to such, and can include other viruses. This term is also intended to include an isolated, recombinant PCV2b divergent ORF2 polypeptide.

The term “antigen” refers to a compound, composition, or immunogenic substance that can stimulate the production of antibodies or a T-cell response, or both, in an animal, including compositions that are injected or absorbed into an animal. The immune response may be generated to the whole molecule, or to a portion of the molecule (*e.g.,* an epitope or hapten). The term “antigen” can include a whole virus, a polypeptide, or a fragment thereof.

As used herein, the term "vaccine composition" includes at least one antigen or immunogen in a pharmaceutically acceptable vehicle useful for inducing an immune response in a host. Vaccine compositions can be administered in dosages, and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration factors such as the age, sex, weight, species and condition of the recipient animal, and the route of administration. The route of administration can be percutaneous, via mucosal administration (e.g., oral, nasal, anal, vaginal) or *via* a parenteral route (intradermal, transdermal, intramuscular, subcutaneous, intravenous, or intraperitoneal). Vaccine compositions can be administered alone, or can be co-administered or sequentially administered with other treatments or therapies. Forms of administration may include suspensions, syrups or elixirs, and preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. Vaccine compositions may be administered as a spray, or mixed in food and/or water, or delivered in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard pharmaceutical texts, such as "Remington's Pharmaceutical Sciences" (1990), may be consulted to prepare suitable preparations, without undue experimentation.

As defined herein, an “immunogenic or immunological composition”, refers to a composition of matter that comprises at least one antigen which elicits an immunological response in the host of a cellular and/or antibody-mediated immune response to the composition or vaccine of interest.

The term "immune response" as used herein refers to a response elicited in an animal. An immune response may refer to cellular immunity (CMI), humoral immunity, or may involve both. The present invention also contemplates a response limited to a part of the immune system. Usually, an “immunological response” includes, but is not limited to, one or more of the following effects: the production or activation of antibodies, B cells, helper T cells, suppressor T cells, and/or cytotoxic T cells and/or yd T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response, such that resistance to new infection will be enhanced, and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time, and/or a lowered viral titer in the infected host.

As used herein, the term "immunogenicity" means capable of producing an immune response in a host animal against an antigen or antigens. This immune response forms the basis of the protective immunity elicited by a vaccine against a specific infectious organism.

An “adjuvant” as used herein means a composition comprised of one or more substances that enhances the immune response to an antigen(s). The mechanism of how an adjuvant operates is not entirely known. Some adjuvants are believed to enhance the immune response by slowly releasing the antigen, while other adjuvants are strongly immunogenic in their own right, and are believed to function synergistically.

As used herein, the term "multivalent" means a vaccine containing more than one antigen, whether from the same microbiological species (e.g., different isolates of *Mycoplasma hyopneumoniae* or PCV), from different species (e.g., isolates from both *Pasteurella hemolytica* and *Pasteurella multocida*), or a vaccine containing a combination of antigens from different genera (for example, a vaccine comprising antigens from *Pasteurella multocida, Salmonella, Escherichia coli, Haemophilus somnus* and *Clostridium*).

The term "pig" or "piglet" as used herein means an animal of porcine origin, while "sow" refers to a female pig of reproductive age and capability. A “gilt” is a female pig who has never been pregnant.

As used herein, the term "virulent" means an isolate that retains its ability to be infectious in an animal host and is capable of causing disease in the host animal.

"Inactivated vaccine" means a vaccine composition containing an infectious organism or pathogen that is no longer capable of replication or growth. The pathogen may be bacterial, viral, protozoal or fungal in origin. Inactivation may be accomplished by a variety of methods, including freeze-thawing, chemical treatment (for example, treatment with β-propiolactone (BPL) or formalin), sonication, radiation, heat, or any other conventional means sufficient to prevent replication or growth of the organism, while maintaining its immunogenicity.

The term “variant” as used herein refers to a polypeptide or a nucleic acid sequence encoding a polypeptide, that has one or more conservative amino acid variations or other minor modifications such that the corresponding polypeptide has substantially equivalent function when compared to the wild-type polypeptide. The term “variant” can also refer to a microorganism comprising a polypeptide or nucleic acid sequence having said variations or modifications as well.

“Conservative variation” denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change, or is another biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine, for another hydrophobic residue, or the substitution of one polar residue with another, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. The term “conservative variation” also includes a substituted amino acid in place of a parent amino acid, provided that antibodies raised to the substituted polypeptide also immunoreact with the parent (unsubstituted) polypeptide.

As used herein, the terms "pharmaceutically acceptable carrier" and "pharmaceutically acceptable vehicle" are interchangeable, and refer to a fluid vehicle for containing vaccine antigens that can be injected into a host without adverse effects. Suitable pharmaceutically acceptable carriers known in the art include, but are not limited to, sterile water, saline, glucose, dextrose, or buffered solutions. Carriers may include auxiliary agents including, but not limited to, diluents, stabilizers (i.e., sugars and amino acids), preservatives, wetting agents, emulsifying agents, pH buffering agents, viscosity enhancing additives, coloring additives, and the like.

"North American PRRS virus" means any PRRS virus having genetic characteristics associated with a North American PRRS virus isolate, such as, but not limited to, the PRRS virus that was first isolated in the United States around the early 1990's (see, e.g., Collins, J. E., et al., 1992, J. Vet. Diagn. Invest. 4:117-126); North American PRRS virus isolate MN-1b (Kwang, J. et al., 1994, J. Vet. Diagn. Invest. 6:293-296); the Quebec LAF-exp91 strain of PRRS virus (Mardassi, H. et al., 1995, Arch. Virol. 140:1405-1418); and North American PRRS virus isolate VR 2385 (Meng, X.-J et al., 1994, J. Gen. Virol. 75:1795-1801). Additional examples of North American PRRS virus strains are known in the art. Genetic characteristics refer to genomic nucleotide sequence similarity and amino acid sequence similarity shared by North American PRRS virus strains. Chinese PRRS virus strains generally evidence about 80-93% nucleotide sequence similarity with North American strains.

"European PRRS virus" refers to any strain of PRRS virus having the genetic characteristics associated with the PRRS virus that was first isolated in Europe around 1991 (see, e.g., Wensvoort, G., et al., 1991, Vet. Q. 13:121-130). "European PRRS virus" is also sometimes referred to in the art as "Lelystad virus". Further examples of European PRRS virus strains are known in the art.

As used herein, a genetically modified virus is "attenuated" if it is less virulent than its unmodified parental strain. A strain is "less virulent" if it shows a statistically significant decrease in one or more parameters determining disease severity. Such parameters may include level of viremia, fever, severity of respiratory distress, severity of reproductive symptoms, or number or severity of pathological lesions, etc.

An "infectious clone" is an isolated or cloned genome of the disease agent (e.g. viruses) that can be specifically and purposefully modified in the laboratory, and then used to re-create the live genetically-modified organism. A live genetically-modified virus produced from the infectious clone can be employed in a live viral vaccine. Alternatively, inactivated virus vaccines can be prepared by treating the live virus derived from the infectious clone with inactivating agents such as formalin, beta-propriolactone, binary ethylenemine or hydrophobic solvents, acids, etc., by irradiation with ultraviolet light or X-rays, by heating, etc.

The present invention provides a vaccine composition for protecting pigs against PCV2, including a highly virulent porcine circovirus type 2b (PCV2b) divergent strain, the composition including a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide comprises Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. As described above, this PCV2b divergent ORF2 polypeptide can further include at least one residue selected from the following: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment, the PCV2b divergent ORF2 polypeptide which includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1, further includes a Lysine (K) at residue 59 and a Lysine (K) at residue 234, according to the numbering of SEQ ID NO: 1.

In a further embodiment, the PCV2b divergent ORF2 polypeptide which includes Leucine (L) at position 89, Threonine (T) at position 90, Aspargine (N) at position 134, a Lysine (K) at residue 59 and a Lysine (K) at residue 234, according to the numbering of

SEQ ID NO: 1, further includes a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment, the PCV2 divergent ORF2 polypeptide is represented by the amino acid sequence of SEQ ID NO: 1 or a fragment thereof. However, the present invention is not limited to this embodiment. For example, in other embodiments, the PCV2 divergent ORF2 polypeptide can be selected from, but is not limited to, the amino acid sequence of SEQ ID NO: 3 or a fragment thereof, the amino acid sequence of SEQ ID NO: 5 or a fragment thereof, the amino acid sequence of SEQ ID NO: 7 or a fragment thereof, the amino acid sequence of SEQ ID NO: 9 or a fragment thereof, the amino acid sequence of SEQ ID NO: 11 or a fragment thereof, the amino acid sequence of SEQ ID NO: 13 or a fragment thereof, the amino acid sequence of SEQ ID NO: 13 or a fragment thereof, the amino acid sequence of SEQ ID NO: 15 or a fragment thereof, the amino acid sequence of SEQ ID NO: 17 or a fragment thereof, the amino acid sequence of SEQ ID NO: 19 or a fragment thereof, the amino acid sequence of SEQ ID NO: 21 or a fragment thereof, the amino acid sequence of SEQ ID NO: 23 or a fragment thereof, the amino acid sequence of SEQ ID NO: 25 or a fragment thereof, the amino acid sequence of SEQ ID NO: 27 or a fragment thereof, the amino acid sequence of SEQ ID NO: 29 or a fragment thereof, the amino acid sequence of SEQ ID NO: 31 or a fragment thereof, the amino acid sequence of SEQ ID NO: 33 or a fragment thereof, the amino acid sequence of SEQ ID NO: 35 or a fragment thereof, the amino acid sequence of SEQ ID NO: 37 or a fragment thereof, the amino acid sequence of SEQ ID NO: 39 or a fragment thereof, the amino acid sequence of SEQ ID NO: 41 or a fragment thereof, the amino acid sequence of SEQ ID NO: 43 or a fragment thereof, the amino acid sequence of SEQ ID NO: 45 or a fragment thereof, the amino acid sequence of SEQ ID NO: 47 or a fragment thereof, the amino acid sequence of SEQ ID NO: 49 or a fragment thereof, the amino acid sequence of SEQ ID NO: 51 or a fragment thereof, the amino acid sequence of SEQ ID NO: 53 or a fragment thereof, or the amino acid sequence of SEQ ID NO: 55 or a fragment thereof.

In one embodiment, the vaccine compositions of the present invention include at least one additional antigen. In one embodiment, the at least one additional antigen is protective against a disease in pigs caused by a microorganism.

In some embodiments, the at least one additional antigen component is protective against a disease in pigs caused by bacteria, viruses, or protozoans that are known to infect pigs. Examples of such microorganisms include, but are not limited to, the following: M. hyo, porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), *Haemophilus parasuis, Pasteurella multocida, Streptococcum suis, Staphylococcus hyicus, Actinobacilllus pleuropneumoniae, Bordetella bronchiseptica, Salmonella choleraesuis, Salmonella enteritidis, Erysipelothrix rhusiopathiae*, *Mycoplama hyorhinis, Mycoplasma hyosynoviae*, leptospira bacteria, *Lawsonia intracellularis*, swine influenza virus (SIV), *Escherichia coli* antigen, *Brachyspira hyodysenteriae*, porcine respiratory coronavirus, Porcine Epidemic Diarrhea (PED) virus, porcine rotavirus (e.g., groups A, B, and C), Torque teno virus (TTV), Porcine Cytomegalovirus, Porcine enteroviruses, Encephalomyocarditis virus, a pathogen causative of Aujesky's Disease, Classical Swine fever (CSF) and a pathogen causative of Swine Transmissable Gastroenteritis, or combinations thereof.

In one embodiment, the at least one additional antigen is *Mycoplasma hyopneumoniae* (M. hyo). In another embodiment, the at least one additional antigen is a PRRS virus, such as a North American PRRS virus strain, a Chinese PRRS virus strain, or a European PRRS virus strain. It is also anticipated that the at least one additional antigen can be a different isolate of PCV2, such as a classical PCV2a strain, a classical PCV2b strain, or other PCV2 genotypes.

In one embodiment, the composition is in the form of an inactivated, PCV2b divergent whole virus that comprises and/or expresses a PCV2b divergent ORF2 polypeptide.

In one embodiment, the ORF2 capsid gene of the PCV2b divergent whole virus corresponds to SEQ ID NO: 2. In a further embodiment, the amino acid sequence of the

PCV2b divergent ORF2 polypeptide which is expressed by the PCV2b divergent whole virus corresponds to SEQ ID NO: 1 or a fragment thereof. However, the present invention is not limited to these embodiments. For example, in some embodiments, the PCV2b divergent ORF2 polypeptide expressed by the PCV2b divergent whole virus can be selected from any of the following sequences or fragments thereof: SEQ ID NO: 3, SEQ ID NO: 5 SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, or SEQ ID NO: 55. The corresponding ORF2 gene sequences are described herein.

In another embodiment, the composition is in the form of an inactivated chimeric virus, wherein the chimeric virus comprises an inactivated recombinant porcine circovirus type 1 that comprises and/or expresses a PCV2b divergent ORF2 polypeptide (chimeric PCV1-2b virus). Chimeric porcine circoviruses and methods for their preparation are described in WO 03/049703 A2, and also in US Patent Nos. 7,279,166 and 7,575,752, which are incorporated herein by reference in their entirety.

In one embodiment, the ORF2 capsid gene of the chimeric PCV1-2 virus corresponds to SEQ ID NO: 2. In a further embodiment, the amino acid sequence of the PCV2b divergent ORF2 polypeptide which is expressed by the chimeric PCV1-2b virus corresponds to SEQ ID NO: 1 or a fragment thereof. However, the present invention is not limited to these embodiments. For example, in some embodiments, the PCV2b divergent ORF2 polypeptide expressed by the chimeric PCV1-2b virus can be selected from any of the following sequences or fragments thereof: SEQ ID NO: 3, SEQ ID NO: 5 SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, or SEQ ID NO: 55.

In yet another embodiment, the composition is in the form of an isolated, recombinant PCV2b divergent ORF2 polypeptide. In one embodiment, the isolated, recombinant PCV2b divergent ORF2 polypeptide is expressed from a vector, such as baculovirus. Alternatively, other known expression vectors can be used, such as including, but not limited to, parapox vectors. In one embodiment, the vector can be a live or inactivated vector.

In a further embodiment, the recombinantly-expressed PCV2b divergent ORF2 polypeptide corresponds to SEQ ID NO: 1 or a fragment thereof. Alternatively, in some embodiments, the recombinantly-expressed PCV2b divergent ORF2 polypeptide can be selected from any of the following or fragments thereof: SEQ ID NO: 3, SEQ ID NO: 5 SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, or SEQ ID NO: 55.

In some forms, immunogenic portions of PCV2 divergent ORF2 protein are used as the antigenic component in the composition. For example, truncated and/or substituted forms or fragments of PCV2 divergent ORF2 protein may be employed in the compositions of the present invention.

It is understood by those of skill in the art that variants of the PCV2b divergent ORF2 polypeptides can be employed in the compositions of the present invention, provided they still retain the antigenic characteristics that render it useful in the vaccine compositions of this invention. Preferably, PCV2b divergent variants have at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% sequence identify with the full-length genomic sequence of the PCV2 isolate termed PCV2B-DIV-MUT . The antigenic characteristics of an immunological composition can be, for example, estimated by the challenge experiment as provided in the Examples. Moreover, the antigenic characteristic of a modified PCV2b divergent ORF2 antigen is still retained when the modified antigen confers at least 70%, preferably 80%, more preferably 90% of the protective immunity as compared to the wild-type PCV2b divergent ORF2 protein having SEQ ID NO: 1.

The PCV2b divergent ORF2 antigen component is provided in the immunogenic/vaccine composition at an antigen inclusion level effective for inducing the desired immune response, namely reducing the incidence of or lessening the severity of clinical signs resulting from infection with a highly virulent PCV2b strain, an example of which is the virus termed PCV2B-DIV-MUT herein. In some embodiments, the composition also provides heterologous protection against classical PCV2a and PCV2b strains.

In one embodiment, a vaccine composition according to the present invention is in the form of an inactivated recombinant porcine circovirus type 1 that comprises and/or expresses a PCV2b divergent ORF2 polypeptide (chimeric PCV1-2bDIV virus). This chimeric virus is included in the compositions of the invention at a level of at least $1.0 \leq RP \leq 5.0$, wherein RP is the Relative Potency unit determined by ELISA antigen quantification (*in vitro* potency test) compared to a reference vaccine. In another embodiment, a chimeric PCV1-2bDIV virus is included in the composition of the invention at a final concentration of about 0.5% to about 5% of 20-times (20X) concentrated bulk PCV1-2bDIV antigen.

In another embodiment, a vaccine composition according to the present invention is in the form of an in the form of an inactivated, PCV2b divergent whole virus that comprises and/or expresses a PCV2b divergent ORF2 polypeptide. This virus is included in the compositions of the invention at a level of at least $1.0 \leq RP \leq 5.0$, wherein RP is the Relative Potency unit determined by ELISA antigen quantification (in vitro potency test) compared to a reference vaccine. In another embodiment, an inactivated PCV2b divergent whole virus is included in the composition of the invention at a final concentration of about 0.5% to about 5% of 20-times (20X) concentrated bulk PCV2b divergent ORF2 antigen.

In yet another embodiment, a vaccine composition according to the present invention is in the form of an isolated, recombinant PCV2b divergent ORF2 polypeptide. The PCV2b divergent ORF2 recombinant protein can be included in the compositions of the invention at a level of at least 0.2 μg antigen/ml of the final immunogenic/vaccine composition (μg/ml). In a further embodiment, the recombinant PCV2b divergent ORF2 polypeptide inclusion level is from about 0.2 to about 400 μg/ml. In yet another embodiment, the recombinant PCV2b divergent ORF2 polypeptide inclusion level is from about 0.3 to about 200 μg/ml. In a still further embodiment, the recombinant PCV2b divergent ORF2 polypeptide inclusion level is from about 0.35 to about 100 μg/ml. In still another embodiment, the recombinant PCV2b divergent ORF2 polypeptide inclusion level is from about 0.4 to about 50 μg/ml.

In one embodiment, a vaccine composition of the present invention includes the combination of a PCV2b divergent ORF2 polypeptide, and at least one M. hyo soluble antigen (e.g., two or more). In one embodiment, a vaccine composition of the invention includes a PCV2b divergent ORF2 polypeptide and one or more of the following M. hyo specific protein antigens: M. hyo proteins of approximately 46kD (p46), 64kD (p64) and 97kD (p97) molecular weights. The M. hyo protein of approximately 64kD (p64) may be alternatively referred to as the p65 surface antigen from M. hyo described by Kim et al. [Infect. Immun. 58(8):2637-2643 (1990)], as well as in U.S. Patent No. 5,788,962. Futo et al. described the cloning and characterization of a 46kD surface protein from M. hyo, which can be employed in the compositions of this invention [J. Bact 177: 1915-1917 (1995)]. Zhang et al. described and characterized a p97 adhesin protein of M. hyo [Infect. Immun. 63: 1013-1019, 1995]. Additionally, King et al. described a 124kD protein termed Mhp1 from the P-5722 strain of M. hyo and presented data suggesting that Mhp1 and p97 are the same protein [Vaccine 15:25-35 (1997)]. Such p97 proteins can be employed in the compositions of this invention. Vaccine compositions of the present invention may include further M. hyo specific protein antigens such as, but not limited to, proteins of approximately 41kD (p41), 42kD (p42), 89kD (p89), and 65kD (p65). *See*, Okada et al., 2000, J. Vet. Med. B 47:527-533 and Kim et al., 1990, Infect. Immun. 58(8):2637-2643. In addition, the M. hyo component can include M. hyo specific protein antigens of approximately102kD (p102) and 216kD (p216). *See*, U.S. Patent Nos. 6,162,435 and 7,419,806 to Minion et al.

In another embodiment, a vaccine composition of the present invention includes the combination of a PCV2b divergent ORF2 polypeptide, at least one M. hyo soluble antigen (e.g., two or more), as well as a PRRS virus antigen. Suitable PRRS virus antigens for use in PCV2b divergent/M. hyo/PRRS compositions of the present invention include North American PRRS virus isolates, Chinese PRRS virus strains, and European PRRS virus strains, as well as genetically modified versions of such isolates/strains. In one embodiment, the PRRS virus antigen component employed in the compositions according to the present invention is a North American PRRS virus.

In some embodiments, the PRRS virus antigen component employed in the compositions of this invention is the North American PRRS virus isolate designated P129 or a live, genetically modified version thereof. Preferably, the genetically modified PRRS virus is unable to produce a pathogenic infection yet is able to elicit an effective immunoprotective response against infection by the wild-type PRRS virus.

A genetically modified PRRS virus for use in the compositions of the invention can be produced from an infectious clone. The preparation of an infectious cDNA clone of the North American PRRS virus isolate designated P129 is described in U.S. Pat. No. 6,500,662 which is hereby incorporated fully by reference. The sequence of P129 cDNA is disclosed in Genbank Accession Number AF494042 and in U.S. Pat. No. 6,500,662.

In one embodiment, a PCV2b divergent/M. hyo combination vaccine is provided as a single-dose, 1-bottle vaccine. In another embodiment, a PCV2b divergent/M. hyo/PRRS virus combination vaccine is provided as a single-dose, 2-bottle vaccine. For example, in some embodiments, a PCV2b divergent/M. hyo combination is provided as a stable liquid composition in a first bottle and a PRRS virus is provided in a lyophilized state in a second bottle. In some embodiments, additional porcine antigens can be added to either the first or the second bottle.

In one embodiment, the PRRS virus component is provided as a lyophilized, genetically modified live virus. Prior to administration, the PCV2b divergent/M. hyo liquid from a first bottle can be used to re-hydrate the PRRS virus in a second bottle so that all three antigens can be administered to the animal in a single-dose.

Vaccines of the present invention can be formulated following accepted convention to include pharmaceutically acceptable carriers for animals, including humans (if applicable), such as standard buffers, stabilizers, diluents, preservatives, and/or solubilizers, and can also be formulated to facilitate sustained release. Diluents include water, saline, dextrose, ethanol, glycerol, and the like. Additives for isotonicity include sodium chloride, dextrose, mannitol, sorbitol, and lactose, among others. Stabilizers include albumin, among others. Other suitable vaccine vehicles and additives, including those that are particularly useful in formulating modified live vaccines, are known or will be apparent to those skilled in the art. See, e.g., Remington's Pharmaceutical Science, 18th ed., 1990, Mack Publishing, which is incorporated herein by reference.

Vaccines of the present invention can further comprise one or more additional immunomodulatory components such as, e.g., an adjuvant or cytokine, among others. Types of suitable adjuvants for use in the compositions of the present invention include the following: an oil-in-water adjuvant, a polymer and water adjuvant, a water-in-oil adjuvant, an aluminum hydroxide adjuvant, a vitamin E adjuvant and combinations thereof. Some specific examples of adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, Corynebacterium parvum, Bacillus Calmette Guerin, aluminum hydroxide gel, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, certain synthetic polymers such as poly amino acids and co-polymers of amino acids, Block copolymer (CytRx, Atlanta, Ga.), QS-21 (Cambridge Biotech Inc., Cambridge Mass.), SAF-M (Chiron, Emeryville Calif.), AMPHIGEN® adjuvant, saponin, Quil A or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant (N,N-dioctadecyl-N',N'-- bis(2-hydroxyethyl)-

propanediamine), "REGRESSIN" (Vetrepharm, Athens, Ga.), paraffin oil, RIBI adjuvant system (Ribi Inc., Hamilton, Mont.), muramyl dipeptide and the like.

Non-limiting examples of oil-in-water emulsions useful in the vaccine of the invention include modified SEAM62 and SEAM 1/2 formulations. Modified SEAM62 is an oil-in-water emulsion containing 5% (v/v) squalene (Sigma), 1% (v/v) SPAN® 85 detergent (ICI Surfactants), 0.7% (v/v) TWEEN® 80 detergent (ICI Surfactants), 2.5% (v/v) ethanol, 200 μg/ml Quil A, 100 μg/ml cholesterol, and 0.5% (v/v) lecithin. Modified SEAM 1/2 is an oil-in-water emulsion comprising 5% (v/v) squalene, 1% (v/v) SPAN® 85 detergent, 0.7% (v/v) Tween 80 detergent, 2.5% (v/v) ethanol, 100 μg/ml Quil A, and 50 μg/ml cholesterol.

Another example of an adjuvant useful in the compositions of the invention is SP-oil. As used in the specification and claims, the term "SP oil" designates an oil emulsion comprising a polyoxyethylene-polyoxypropylene block copolymer, squalane, polyoxyethylene sorbitan monooleate and a buffered salt solution. Polyoxyethylene-polyoxypropylene block copolymers are surfactants that aid in suspending solid and liquid components. These surfactants are commercially available as polymers under the trade name Pluronic®. The preferred surfactant is poloxamer 401 which is commercially available under the trade name Pluronic® L-121. In general, the SP oil emulsion is an immunostimulating adjuvant mixture which will comprise about 1 to 3% vol/vol of block copolymer, about 2 to 6% vol/vol of squalane, more particularly about 3 to 6% of squalane, and about 0.1 to 0.5% vol/vol of polyoxyethylene sorbitan monooleate, with the remainder being a buffered salt solution. In one embodiment, the SP-oil emulsion is present in the final composition in v/v amounts of about 1% to 25%, preferably about 2% to 15%, more preferably about 5% to 12% v/v.

Yet another example of a suitable adjuvant for use in the compositions of the invention is AMPHIGEN™ adjuvant which consists of de-oiled lecithin dissolved in an oil, usually light liquid paraffin.

Other examples of adjuvants useful in the compositions of the invention are the following proprietary adjuvants: Microsol Diluvac Forte® duel emulsion adjuvant system, Emunade adjuvant, and Xsolve adjuvant. Both the Emunade and Xsolve adjuvants are emulsions of light mineral oil in water, but Emunade also contains alhydrogel, and d,l-α-tocopheryl acetate is part of the XSolve adjuvant. A still further example of a suitable adjuvant for use in the compositions of the invention is ImpranFLEX™ adjuvant (a water-in-oil adjuvant). A still further example of a suitable adjuvant is a Carbomer (Carbopol®) based adjuvant. Preferred Carbopol® adjuvants include Carbopol® 934 polymer and Carbopol®941 polymer.

In one embodiment, the adjuvant or adjuvant mixture is added in an amount of about 100 μg to about 10 mg per dose. In another embodiment, the adjuvant/adjuvant mixture is added in an amount of about 200 μg to about 5 mg per dose. In yet another embodiment, the adjuvant/adjuvant mixture is added in an amount of about 300 μg to about 1 mg/dose.

The adjuvant or adjuvant mixture is typically present in the vaccine composition of the invention in v/v amounts of about 1% to 25%, preferably about 2% to 15%, more preferably about 5% to 12% v/v.

Other "immunomodulators" that can be included in the vaccine include, e.g., one or more interleukins, interferons, or other known cytokines. In one embodiment, the adjuvant may be a cyclodextrin derivative or a polyanionic polymer, such as those described in U.S. Pat. Nos. 6,165,995 and 6,610,310, respectively.

The present invention also provides a method of immunizing a pig against a PCV2b divergent strain, the method including administering to the pig a composition according to the present invention, as described above. This composition for administration includes a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. As described above, this PCV2b divergent ORF2 polypeptide can further include at least one residue selected from the following: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment, the composition for administration includes a virus comprising and/or expressing the PCV2b divergent ORF2 polypeptide. In another embodiment, the composition for administration includes an isolated, recombinant PCV2b ORF2 polypeptide.

In one embodiment of the method of the present invention, the composition is administered intramuscularly, intradermally, transdermally, subcutaneously, or orally. In another embodiment, the composition is administered in a single dose. In yet another embodiment, the composition is administered as two doses.

In a further embodiment, the composition is administered to pigs having maternally-derived antibodies against PCV2.

In one embodiment, the composition is administered to pigs at 3 weeks of age or older.

Vaccine compositions according to the present invention can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species and condition of the recipient animal, and the route of administration. The route of administration can be percutaneous, *via* mucosal administration (e.g., oral, nasal, anal, vaginal) or *via* a parenteral route (intradermal, transdermal, intramuscular, subcutaneous, intravenous, or intraperitoneal). Vaccine compositions according to the present invention can be administered alone, or can be co-administered or sequentially administered with other treatments or therapies. Forms of administration may include suspensions, syrups or elixirs, and preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. Vaccine compositions according to the present invention may be administered as a spray, or mixed in food and/or water, or delivered in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired.

The present invention further provides a kit. This kit includes a bottle containing a vaccine composition according to the present invention for protecting pigs against a highly virulent porcine circovirus type 2b (PCV2b) divergent strain. This vaccine composition includes a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide includes Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1. As described above, this PCV2b divergent ORF2 polypeptide can further include at least one residue selected from the following: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215, according to the numbering of SEQ ID NO: 1.

In one embodiment of the kit, the vaccine composition is in the form of a virus comprising and/or expressing the PCV2b divergent ORF2 polypeptide. In another embodiment of the kit, the vaccine composition is in the form of an isolated, recombinant PCV2b divergent ORF2 polypeptide.

In one embodiment of the kit of the present invention, the vaccine composition in the bottle is provided as a ready-to-use liquid composition. In another embodiment of the kit, the vaccine composition in the bottle is provided in a lyophilized form. In a further embodiment, the kit can include a diluent. In yet another embodiment, the kit can further include an instruction manual which contains the information for administration of the vaccine composition.

Another aspect of the present invention provides methods of producing a vaccine composition which is in the form of an inactivated chimeric virus, wherein the chimeric virus includes an inactivated recombinant porcine circovirus type 1 that expresses a PCV2b divergent ORF2 polypeptide. Chimeric porcine circoviruses and methods for their preparation are described in WO 03/049703 A2, and also in US Patent Nos. 7,279,166 and 7,575,752. Methods of producing a chimeric porcine circovirus including an inactivated PCV1 that expresses a PCV2b divergent ORF2 polypeptide are described in Example 1 below. In one embodiment, the final composition is prepared by combining the inactivated cPCV1-2b virus with a suitable adjuvant and/or other pharmaceutically acceptable carrier.

A further aspect of the present invention provides methods of producing a vaccine composition which is in the form of an inactivated, PCV2b divergent whole virus that expresses PCV2b divergent ORF2 polypeptide. Such methods are described in Example 3 below. In one embodiment, the final composition is prepared by combining the inactivated PCV2B-DIV-MUT virus with a suitable adjuvant and/or other pharmaceutically acceptable carrier.

Yet another aspect of the present invention provides methods of producing recombinant PCV2 divergent ORF2 protein, i) by permitting infection of susceptible cells in culture with a recombinant viral vector containing PCV2 divergent ORF2 DNA coding sequences, wherein ORF2 protein is expressed by the recombinant viral vector, and ii) thereafter recovering the ORF2 protein in the supernatant. Typically, high amounts of PCV2 divergent ORF2 protein can be recovered in the supernantant. High amounts of PCV2 divergent ORF2 means more than about 20 μg/mL supernate, preferably more than about 25 μg/mL, even more preferred more than about 30 μg/mL, even more preferred more than about 40 μg/mL, even more preferred more than about 50 μg/mL, even more preferred more than about 60 μg/mL, even more preferred more than about 80 μg/mL, even more preferred more than about 100 μg/mL, even more preferred than about 150 μg/mL, most preferred than about 190 μg/mL.

Preferred cell cultures have a cell count between about 0.3-2.0x10^6 cells/mL, more preferably from about 0.35-1.9x10^6 cells/mL, still more preferably from about 0.4-1.8x10^6 cells/mL, even more preferably from about 0.45-1.7x10^6 cells/mL, and most preferably from about 0.5-1.5x10^6 cells/mL. Preferred cells are determinable by those of skill in the art. Preferred cells are those susceptible for infection with an appropriate recombinant viral vector, containing a PCV2 divergent ORF2 DNA and expressing the PCV2 divergent ORF2 protein. Preferably the cells are insect cells, and more preferably, they include the insect cells sold under the trademark Sf+ insect cells (Protein Sciences Corporation, Meriden, Conn.).

Appropriate growth media will also be determinable by those of skill in the art with a preferred growth media being serum-free insect cell media such as Excell 420 (JRH Biosciences, Inc., Lenexa, Kans.) and the like. Preferred viral vectors include baculovirus such as BaculoGold (BD Biosciences Pharmingen, San Diego, Calif.), in particular if the production cells are insect cells. Although the baculovirus expression system is preferred, it is understood by those of skill in the art that other expression systems will work for purposes of the present invention, namely the expression of PCV2 divergent ORF2 into the supernatant of a cell culture. Such other expression systems may require the use of a signal sequence in order to cause ORF2 expression into the media. However, when ORF2 is produced by a baculovirus expression system, then typically it does not require any signal sequence or further modification to cause expression of ORF2 into the media. It is believed that this protein can independently form virus-like particles (Journal of General Virology 2000, Vol. 81, pp. 2281-2287), and be secreted into the culture supernatant. The recombinant viral vector containing the PCV2 divergent ORF2 DNA sequences has a preferred multiplicity of infection (MOI) of between about 0.03-1.5, more preferably from about 0.05-1.3, still more preferably from about 0.09-1.1, and most preferably from about 0.1-1.0, when used for the infection of the susceptible cells. Preferably the MOIs mentioned above relates to one mL of cell culture fluid. Preferably, the method described herein comprises the infection of 0.35-1.9x 10^6 cells/mL, still more preferably of about 0.4-1.8x10^6 cells/mL, even more preferably of about 0.45-1.7x10^6 cells/mL, and most preferably of about 0.5-1.5x10^6 cells/mL with a recombinant viral vector containing a PCV2 divergent ORF2 DNA and expressing the PCV2 divergent ORF protein having a MOI (multiplicity of infection) of between about 0.03-1.5, more preferably from about 0.05-1.3, still more preferably from about 0.09-1.1, and most preferably from about 0.1-1.0.

The infected cells are then incubated over a period of up to ten days, more preferably from about two days to about ten days, still more preferably from about four days to about nine days, and most preferably from about five days to about eight days. Preferred incubation conditions include a temperature between about 22-32°C., more preferably from about 24-30°C, still more preferably from about 25-29°C, even more preferably from about 26-28°C, and most preferably about 27°C. Preferably, the Sf+ cells are observed following inoculation for characteristic baculovirus-induced changes. Such observation may include monitoring cell density trends and the decrease in viability during the post-infection period. Peak viral titer is typically observed 3-5 days after infection, and peak ORF2 release from the cells into the supernatant is typically obtained between days 5 and 8, and/or when cell viability decreases to less than 10%.

The recovery process preferably begins with the separation of cell debris from the expressed PCV2 divergent ORF2 polypeptide in media via a separation step. Preferred separation steps include filtration, centrifugation at speeds up to about 20,000 x g, continuous flow centrifugation, chromatographic separation using ion exchange or gel filtration, and conventional immunoaffinity methods. Those methods are known to persons skilled in the art (e.g. Harris and Angel (eds.), Protein purification methods--a practical approach, IRL press Oxford 1995). Preferred filtration methods include dead-end microfiltration and tangential flow (or cross flow) filtration, including hollow fiber filtration. Of these, dead-end microfiltration is preferred. Preferred pore sizes for dead-end microfiltration are between about 0.30-1.35μm, more preferably between about 0.35-1.25μm, still more preferably between about 0.40-1.10μm, and most preferably between about 0.45-1.0μm.

For recovery of recombinant PCV2 divergent ORF2 polypeptide that will be used in an immunogenic or immunological composition such as a vaccine, the inclusion of an inactivation step is preferred in order to inactivate the viral vector. Preferably, this inactivation is done either just before or just after the filtration step, with after the filtration step being the preferred time for inactivation. Any conventional inactivation method can be used for purposes of the present invention. Thus, inactivation can be performed by chemical and/or physical treatments. In preferred forms, the volume of harvest fluids is determined and the temperature is brought to between about 32-42°C, more preferably between about 34-40°C, and most preferably between about 35-39°C. Preferred inactivation methods include the addition cyclized binary ethylenimine (BEI), preferably in a concentration of about 1 to about 20 mM, preferably of about 2 to about 10 mM, still more preferably of about 2 to about 8 mM, still more preferably of about 3 to about 7 mM, most preferably of about 5 mM. For example the inactivation includes the addition of a solution of 2-bromoethyleneamine hydrobromide, preferably of about 0.4M, which has been cyclized to 0.2M binary ethylenimine (BEI) in 0.3N NaOH, to the fluids to give a final concentration of about 5 mM BEI. Preferably, the fluids are then stirred continuously for 72-96 hours, and the inactivated harvest fluids can be stored frozen at -40°C or below or between about 1-7°C. After inactivation is completed, a sodium thiosulfate solution, preferably at 1.0M, is added to neutralize any residual BEI. Preferably, the sodium thiosulfate is added in equivalent amount as compared to the BEI added prior to for inactivation. For example, in the event BEI is added to a final concentration of 5 mM, a 1.0 M sodium thiosulfate solution is added to give a final minimum concentration of 5 mM to neutralize any residual BEI.

A further aspect of the present invention relates to a method for preparing a composition comprising PCV2 divergent ORF2 protein, and inactivated viral vector. This method includes the steps: i) cloning the amplified PCV2 divergent ORF2 gene into a transfer vector; ii) transfecting the portion of the transfer vector containing the recombinant PCV2 divergent ORF2 gene into a virus; iii) infecting cells in media with the transfected viral vector; iv) causing the transfected viral vector to express the PCV2 divergent ORF2 recombinant protein from PCV2 divergent ORF2 gene; v) separating cells from the supernatant; vi) recovering the expressed PCV2 divergent ORF2 protein from the supernatant; and vii) inactivating the recombinant viral vector. Preferably, the recombinant viral vector is a baculovirus-containing ORF2 DNA coding sequences, and the cells are Sf+ cells. Preferred separation steps are those described above, most preferred is the filtration step. Preferred inactivation steps are those described above. Preferably, inactivation is performed between about 35-39°C and in the presence of 2 to 8 mM BEI, still more preferred in the presence of about 5 mM BEI. Preferably, inactivation is performed for at least 24 hours, even more preferred for 24 to 72 hours.

According to a further aspect, the method for preparing a composition comprising PCV2 divergent ORF2 protein, and inactivated viral vector, as described above, also includes an neutralization step after step vii). This step viii) comprises adding of an equivalent amount of an agent that neutralizes the inactivation agent within the solution. Preferably, if the inactivation agent is BEI, addition of sodium thiosulfate to an equivalent amount is preferred. Thus, according to a further aspect, step viii) comprises adding of a sodium thiosulfate solution to a final concentration of about 1 to about 20 mM, preferably of about 2 to about 10 mM, still more preferably of about 2 to about 8 mM, still more preferably of about 3 to about 7 mM, most preferably of about 5 mM, when the inactivation agent is BEI.

In another aspect of the present invention, a method for preparing a composition, preferably an antigenic composition, such as for example a vaccine, for invoking an immune response against a PCV2 divergent strain is provided. Generally, this method includes the steps of transfecting a construct into a virus, wherein the construct comprises i) recombinant DNA from ORF2 of a PCV2 divergent strain, ii) infecting cells in growth media with the transfected virus, iii) causing the virus to express the recombinant protein from PCV2 divergent ORF2, iv) recovering the expressed ORF2 protein from the supernatant, v) and preparing the composition by combining the recovered protein with a suitable adjuvant and/or other pharmaceutically acceptable carrier.

The following examples set forth preferred materials and procedures in accordance with the present invention. However, it is to be understood that these examples are provided by way of illustration only, and nothing therein should be deemed a limitation upon the overall scope of the invention.

Example 1: Chimeric Porcine Circovirus (cPCV)1-2 Production Methods

The cPCV1-2 is constructed by cloning the immunogenic capsid gene of a pathogenic porcine circovirus type 2b divergent strain (termed "PCV2B-DIV-MUT") into the genomic backbone of the nonpathogenic porcine circovirus type 1 (PCV1). The procedure for construction of the chimeric DNA clone is described, for example, in US Patent No. 7,279,166, which is incorporated herein by reference in its entirety. An infectious stock of the chimeric virus is used to infect Porcine Kidney (PK)-15 cells grown in Minimum Essential Medium (MEM) supplemented with 0.05% lactalbumin hydrolysate (LAH), 30 µg/mL gentamicin sulfate, and 5% fetal bovine serum. The resulting cPCV1-2 infected PK-15 cells is further expanded by serial passing four more times using the same growth medium, except with 2-3% fetal bovine serum. The fifth passage is frozen, thawed and filtered, and the resulting lysates are used to prepare a pre-master seed and subsequent master seed.

The medium which is used for producing virus seeds is the same as that used in producing virus stock. For the growth medium, MEM, OptiMEM, or equivalent is the basal medium which can be used for planting the PK-15 cell line for outgrowth. The growth medium can be supplemented with up to 10% bovine serum, up to 0.5% lactalbumin hydrolysate, up to 0.5% bovine serum albumin, and up to 30 µg/mL gentamicin. For the virus propagation medium, MEM, OptiMEM, or equivalent is used. The virus propagation medium can be supplemented with up to 0.5% lactalbumin hydrolysate, up to 2% bovine serum, up to 0.5% bovine serum albumin, and up to 30 µg/mL gentamicin. Up to 5 g/L glucose and up to 5 mmol/L L-glutamine can be added to the growth medium and/or the virus propagation medium as required to sustain the cells.

The cPCV1-2 master seed virus are added to a cell suspension of PK-15 cells and adsorbed for up to 3 hours. Seed virus is diluted in growth basal medium to provide a multiplicity of infection (MOI) of 0.1 to 0.2.

Cultures of PK-15 cells are initially inoculated with working seed virus at the time of cell planting, or when cells reach approximately 20% to 50% confluency. This initial passage may be referred as "One-Step Infection Method" for the production of antigen stock, or may be further used for serial passages. For serial passages, the cPCV1-2 infected PK-15 cells are further expanded up to passage 7 by serial splits at the ratio of 1:5-20 for virus propagation. Culture medium containing an infected cell suspension from the previous passage can serve as seed material for the next passage. The cPCV1-2 infected cells are incubated for three (3) to 14 days for each passage at 36 ± 2°C when cells reach ≥ 90% confluency. The cPCV1-2 virus causes observable cytopathic changes during viral replication. At harvest, rounding of cells and considerable floating debris is observed. Cultures are also observed for visual evidence of bacterial or fungal contamination. The incubation time between harvests for the cPCV antigen is provided in Table 1 below:

Table 1 Minimum and Maximum Times for Harvesting cPCV Antigen

Method	Minimum / Maximum Time	Temperature Range
One-Step Infection	5 to 16 days	36 ± 2°C
Serial Passage (MSV + 1 to MSV + 4)	16 to 36 Days	36 ± 2°C

The cPCV1-2 culture fluids are harvested into sterile vessels and are sampled for mycoplasmal contamination using known methods. Multiple harvests may be conducted from roller bottles, bioreactors and perfusion vessels.

Prior to inactivation of the harvested cPCV1-2 virus, one or more antigen lots may be concentrated (e.g., up to 60X) by ultrafiltration. The concentrates may be washed with balanced salt solution to reduce serum proteins.

The method of inactivation, attenuation, or detoxification of the cPCV1-2 virus will now be described. After cPCV antigen concentration, β-propiolactone (BPL) is added to the pooled cPCV1-2 viral material to obtain an approximate concentration of 0.2% v/v. The pooled viral fluids are then agitated for a minimum of 15 minutes and then the inactivating bulk antigen fluids are transferred to a second sterile vessel. The transferred antigen fluids are maintained at 2 - 7°C, with constant agitation, for a minimum of 24 hours. After a minimum of 24 hours, a second addition of 0.2% v/v of BPL is added to the pooled suspension. The contents are subsequently agitated, transferred to a third vessel, and maintained at 2 - 7°C, with constant agitation, for an additional time of not less than 84 hours. In general, the total inactivation time is not less than 108 hours and not more than 120 hours. The inactivation method is summarized in Table 2 below.

Table 2 Inactivation Method

Inactivant	Final Concentration	Temp. Range	Time-Hours (Min/Max)
β-propiolactone (BPL)	0.4% v/v(2 x 0.2% v/v additions)	2 – 7°C (w/Agitation)	108 - 120

The inactivation is terminated by the addition of a final concentration of not more than 0.1 M solution of sodium thiosulfate. The pH of the inactivated antigen stock is adjusted to about 6.8 using NaOH or HCl. Following inactivation, a representative sample is taken from the pool and tested for completion of inactivation. The inactivated cPCV1-2 antigen product is standardized to a meet a target of greater than 1.0 RP as measured via potency ELISA. In one embodiment, the final composition is prepared by combining the inactivated cPCV1-2b virus with a suitable adjuvant and/or other pharmaceutically acceptable carrier.

Example 2: Methods of Producing Recombinant PCV2b Divergent Capsid Protein

Production of the subunit vaccine is the result of a two phase process: firstly, the production of the ORF2 subunit antigen in the baculovirus expression system and secondly, the formulation/manufacturing of the final product. For the initial steps (the construction of the recombinant baculovirus and the production of the ORF2 antigen), the basic technology process used will now be described. A baculovirus expression system is used for expression of the ORF2 gene from a PCV2b divergent strain. The recombinant baculovirus containing the PCV2 ORF2 gene is generated as follows: viral DNA is isolated from PK-15 cells infected with the PCV2b divergent strain identified herein as "PCV2B-DIV-MUT". The ORF2 gene from this PCV2b divergent strain is PCR amplified to contain a 5' Kozak's sequence (ccgccatg) and a 3' *Eco*R1 site (gaattc), and is cloned into the pGEM-T-Easy vector (Promega, Madison, Wis.). Then, it is subsequently excised and subcloned into the transfer vector pVL1392 (BD Biosciences Pharmingen, San Diego, Calif.). The pVL1392 plasmid containing the PCV2b divergent ORF2 gene is then co-transfected with BaculoGold®. (BD Biosciences Pharmingen) baculovirus DNA into *Spodoptera frugiperda* (Sf+) insect cells (Protein Sciences, Meriden, Conn.) to generate the recombinant baculovirus containing the PCV2b divergent ORF2 gene. The recombinant baculovirus containing this PCV2b divergent ORF2 gene is plaque-purified and Master Seed Virus (MSV) is propagated on the SF+ cell line, aliquoted, and stored at -70°C. The MSV is positively identified as PCV2 ORF2 baculovirus by PCR-RFLP using baculovirus-specific primers. Insect cells infected with PCV2 ORF2 baculovirus to generate MSV or Working Seed Virus express the PCV2 ORF2 antigen. Expression of the ORF2 gene of PCV2B-DIV-MUT is confirmed by an immunoassay using hyperimmune serum raised against PCV2B-DIV-MUT in rabbits, or monoclonal antibodies, in an indirect fluorescent antibody assay. Alternatively, expression of the ORF2 gene of PCV2B-DIV-MUT is confirmed by an immunoassay using an antibody raised against classical PCV2a or PCV2b that cross reacts with the PCV2b divergent strain. Additionally, the identity of the PCV2b divergent ORF2 baculovirus is confirmed by N-terminal amino acid sequencing. The PCV2b divergent ORF2 baculovirus MSV is also tested for purity in accordance with 9 C.F.R. 113.27 (c), 113.28, and 113.55.

The recombinant viral vector containing the PCV2 ORF2 DNA sequences has a preferred multiplicity of infection (MOI) of between about 0.03-1.5, more preferably from about 0.05-1.3, still more preferably from about 0.09-1.1, and most preferably from about 0.1-1.0, when used for the infection of the susceptible cells. Preferably, the method described herein comprises the infection of 0.35-1.9 $x10^6$ cells/mL, still more preferably of about 0.4-1.8 $x10^6$ cells/mL, even more preferably of about 0.45-1.7 $x10^6$ cells/mL, and most preferably of about 0.5-1.5 x 10^6 cells/mL with a recombinant viral vector containing a PCV2 ORF2 DNA and expressing the PCV2 ORF protein having a MOI (multiplicity of infection) of between about 0.03-1.5, more preferably from about 0.05-1.3, still more preferably from about 0.09-1.1, and most preferably from about 0.1-1.0.

The infected cells are then incubated over a period of up to ten days, more preferably from about two days to about ten days, still more preferably from about four days to about nine days, and most preferably from about five days to about eight days. Preferred incubation conditions include a temperature between about 22-32°C, more preferably from about 24-30°C, still more preferably from about 25-29°C, even more preferably from about 26-28°C, and most preferably about 27°C. Preferably, the Sf+ cells are observed following inoculation for characteristic baculovirus-induced changes. Such observation may include monitoring cell density trends and the decrease in viability during the post-infection period. A peak viral titer is typically observed 3-5 days after infection and peak ORF2 release from the cells into the supernatant is typically obtained between days 5 and 8, and/or when cell viability decreases to less than 10%.

In one embodiment, a 1000 mL spinner flask is seeded with approximately $1.0x10^6$ Sf+ cells/ml in 300 mL of Excell 420 media. The flask is then incubated at 27°C and agitated at 100 rpm. Subsequently, the flask is seeded with PCV2b divergent ORF2/Bac (recombinant baculovirus containing the PCV2b divergent ORF2 gene) virus seed with a 0.1 MOI after 24 hours of incubation.

The flask is then incubated at 27°C for a total of 6 days. After incubation, the contents of the flask are centrifuged, and the resulting supernatant is harvested, microfiltered through a 0.45-1.0 μm pore size membrane, and then inactivated. The supernatant is inactivated by bringing its temperature to 37+/-2°C, and adding 10 mM binary ethlylenimine (BEI) to the supernatant. The supernatant is then stirred continuously for 48 hrs. A 1.0 M sodium thiosulfate solution to give a final minimum concentration of 5 mM is added to neutralize any residual BEI. The quantity of ORF2 in the neutralized supernatant is then quantified using an ELISA assay procedure such as the one described in Example 1 of US Patent No. 7,700,285 to Eichmeyer et al. The detection antibody used is a monoclonal antibody to PCV2b divergent ORF2 capsid protein.

The present invention is scalable from small scale production of recombinant PCV2b divergent ORF2 to large scale production of recombinant PCV2b divergent ORF2.

The second phase of the vaccine production is the formulation/manufacturing of the final product. The blending strategy is based on: a) a fixed antigen content per dose, and b) a fixed amount of at least one adjuvant. In one embodiment, the pharmaceutical form of the finished product is equivalent to an oil-in-water emulsion. In order to prepare the final vaccine, the adjuvant is added to the antigenic fraction and stirred until a homogeneous emulsion is obtained. Evidence is provided of satisfactory homogeneity. To ensure that a batch of vaccine will lead to the claimed efficacy, its relative potency is determined by an *in vivo* assay which has been validated. Based on the analysis performed, the potency test is able to detect sub-potent batches.

Example 3: Methods of Producing Inactivated PCV2b divergent whole virus

An infectious stock of the PCV2b divergent virus: PCV2B-DIV-MUT is used to infect Porcine Kidney (PK)-15 cells grown in Minimum Essential Medium (MEM), supplemented with 0.05% lactalbumin hydrolysate (LAH), 30 μg/mL gentamicin sulfate, and 5% fetal bovine serum. The resulting PCV2B-DIV-MUT infected PK-15 cells are further expanded by serial passing four more times using the same growth medium, except with 0.5-3% fetal bovine serum. The fifth passage is frozen, thawed and filtered, and the resulting lysates are used to prepare a pre-master seed and subsequent master seed.

The medium which is used for producing virus seeds is the same as that used in producing virus stock. For the growth medium, MEM, OptiMEM, or equivalent is the basal medium which can be used for planting the PK-15 cell line for outgrowth. The growth medium can be supplemented with up to 10% bovine serum, up to 0.5% lactalbumin hydrolysate, up to 0.5% bovine serum albumin, and up to 30 μg/mL gentamicin. For the virus propagation medium, MEM, OptiMEM, or equivalent is used. The virus propagation medium can be supplemented with up to 0.5% lactalbumin hydrolysate, up to 2% bovine serum, up to 0.5% bovine serum albumin, and up to 30 μg/mL gentamicin. Up to 5 g/L glucose and up to 5 mmol/L L-glutamine can be added to the growth medium and/or the virus propagation medium, as required to sustain the cells.

The PCV2B-DIV-MUT master seed virus is added to a cell suspension of PK-15 cells and adsorbed for up to 3 hours. Seed virus is diluted in growth basal medium to provide a multiplicity of infection (MOI) of 0.1 - 0.2.

Cultures of PK-15 cells are initially inoculated with working seed virus at the time of cell planting, or when cells reach approximately 20% to 50% confluency. This initial passage may be referred as "One-Step Infection Method" for the production of antigen stock, or may be further used for serial passages. For serial passages, the infected PCV2B-DIV-MUT PK-15 cells are further expanded up to passage 7 by serial splits at the ratio of 1:5-20 for virus propagation. Culture medium containing an infected cell suspension from the previous passage can serve as seed material for the next passage. The PCV2B-DIV-MUT infected cells are incubated for three (3) to 14 days for each passage at 36 ± 2°C when cells reach ≥ 90% confluency. The PCV2B-DIV-MUT virus can cause observable cytopathic changes during viral replication. At harvest, rounding of cells and considerable floating debris is observed. Cultures are also observed for visual evidence of bacterial or fungal contamination. The incubation times between harvests for the PCV2B-DIV-MUT antigen are the same as those provided in Table 1 above.

The PCV2B-DIV-MUT culture fluids are harvested into sterile vessels, and are sampled for mycoplasmal contamination using known methods. Multiple harvests may be conducted from roller bottles, bioreactors and perfusion vessels.

Prior to inactivation of the harvested PCV2B-DIV-MUT virus, one or more antigen lots may be concentrated (e.g., up to 60X) by ultrafiltration. The concentrates may be washed with balanced salt solution to reduce serum proteins.

The method of inactivation, attenuation, or detoxification of the PCV2B-DIV-MUT virus is the same as that described in Example 1 and Table 2 above. The inactivation is terminated by the addition of a final concentration of not more than 0.1 M solution of sodium thiosulfate. The pH of the inactivated antigen stock is adjusted to about 6.8 using NaOH or HCl. Following inactivation, a representative sample is taken from the pool and tested for completion of inactivation. The inactivated PCV2B-DIV-MUT antigen product is standardized to a meet a target of greater than 1.0 RP, as measured via potency ELISA. In one embodiment, the final composition is prepared by combining the inactivated PCV2B-DIV-MUT virus with a suitable adjuvant and/or other pharmaceutically acceptable carrier.

Example 4: PCV2b Proof of Principle Study

The objective of the study was to assess a PCV2b divergent candidate vaccine for homologous and heterologous protection. The study design is outlined in Table 3. The IVP for T04, T08 and T12 consisted of a killed PCV2b-divergent virus, adjuvanted with 10% SP-Oil. The IVP for T02, T06 and T10 consisted of a killed chimeric PCV1:2a virus, adjuvanted with 10% SP-Oil. The IVP for T03, T07 and T11 consisted of a killed chimeric PCV1:2b virus, adjuvanted with 10% SP-Oil.

Table 3 Proof of Principle Study Design

<table>
<tr><th rowspan="2">Group</th><th rowspan="2">N</th><th rowspan="2">Investigational Veterinary Product (IVP)</th><th rowspan="2">Vaccinations</th><th colspan="3">Challenge</th><th rowspan="2">Necropsy</th></tr>
<tr><th>Day</th><th>Strain</th><th>Dose, Route</th></tr>
<tr><td>T01</td><td>10</td><td>Placebo</td><td rowspan="12">Day 0 (~3 wks of age) 2 mL IM Left side of neck</td><td rowspan="12">Day 21 (~6 wks of age)</td><td rowspan="4">PCV2b</td><td rowspan="12">1 mL/ IM 2 mL/ IN</td><td rowspan="12">Necropsy (~9 wks of age) and Tissue Collection</td></tr>
<tr><td>T02</td><td>10</td><td>Chimeric PCV1:PCV2a</td></tr>
<tr><td>T03</td><td>10</td><td>Chimeric PCV1:PCV2b</td></tr>
<tr><td>T04</td><td>10</td><td>Killed PCV2b-Divergent</td></tr>
<tr><td>T05</td><td>10</td><td>Placebo</td><td rowspan="4">PCV2a</td></tr>
<tr><td>T06</td><td>10</td><td>Chimeric PCV1:PCV2a</td></tr>
<tr><td>T07</td><td>10</td><td>Chimeric PCV1:PCV2b</td></tr>
<tr><td>T08</td><td>10</td><td>Killed PCV2b-Divergent</td></tr>
<tr><td>T09</td><td>10</td><td>Placebo</td><td rowspan="4">PCV2b-Divergent</td></tr>
<tr><td>T10</td><td>10</td><td>Chimeric PCV1:PCV2a</td></tr>
<tr><td>T11</td><td>10</td><td>Chimeric PCV1:PCV2b</td></tr>
<tr><td>T12</td><td>10</td><td>Killed PCV2b-Divergent</td></tr>
</table>

Pigs were between 3 and 4 weeks of age on Day 0 for vaccination. A single dose of 2 mL of the assigned vaccine was administered intramuscularly (IM) into the right side of the neck. A single 3 mL sterile syringe with 1” or ¾” needle was used for each pig. Vaccination details were recorded. Pigs were observed within 1 hour (± 30 minutes) after each vaccination for abnormal clinical signs, including but not limited to: lethargy, labored breathing, vomiting, and incoordination. Any observed clinical signs were documented on the general health form. A veterinarian was notified to follow up on two pigs which presented with signs of overall poor condition and declining health. Those animals were humanely euthanized.

Challenge was conducted on Day 21, when the pigs are about 6-7 weeks of age. Each pig was inoculated with a total 3 mL of respective challenge virus, pre-diluted to 4.8-5.8 log10 TCID50 /mL, with 1 mL administered intranasally (IN) in each nostril, and

1 mL administered intramuscularly (IM). A reserved aliquot of the challenge viruses was titrated following the challenge to confirm the actual challenge dose.

Individual blood samples (5-10 mL) were collected in serum separator tubes (SST) on Day -1 (prior to vaccination), and Days 7, 14, 20/21, 28, 35, and 42. Samples were aliquoted and stored at ≤ -65°C, and later tested for PCV2 antibody titers by ELISA, and PCV2 viremia by qPCR.

Individual fecal swabs were also taken from each pig prior to challenge (Day 20/21), and weekly post-challenge. Individual sterile polyester swabs were used for collecting fecal swab, and placed in a tube containing 3 mL sterile PBS medium. Swabs were swirled for 5 seconds in the medium before discarded. Samples were aliquoted and stored at ≤ -65°C. The fecal swab samples were tested for virus shedding by standard quantitative PCR procedure.

During necropsy, sections of tracheobronchial, mesentery and superficial inguinal lymph nodes, and tonsil tissues were also collected in duplicate for each pig, individually identified, and fixed in 10% buffered formalin. One set was archived, while the other was submitted for standard histopathology examination for lymphoid depletion (PCVAD), and histiocytic replacement. The conclusion was recorded as Yes (+) or No (-). A pig was considered having lymphoid depletion or histiocytic replacement if one or more tissues were scored "+". In addition, the tissues were also tested for PCV2 antigen by IHC. The results were recorded as 0 (no staining) and 1-3 (different levels of staining). A score 0 was considered as PCV2 IHC (-), and a score of 1 or higher was considered as PCV2 IHC (+). A pig was considered IHC (+) if one or more tissues were IHC (+).

The primary outcomes were the homologous and heterologous protection of a candidate vaccine when compared to the placebo. The primary variable was viremia, and the secondary variables were fecal shedding and histopathological lesions.

The results indicated that pigs remained negative for PCV2 viremia and fecal shed prior to challenge, as indicated in Tables 4 and 5. Throughout the study, however, all pigs in all treatment groups became positive at some point for PCV2, as assessed by quantitative PCR for PCV2 viremia (Table 4) and PCV2 fecal shedding (Table 5).

Table 4 PCV2 Viremia

Trt	Challenge	Time Point (Geometric LS Mean DNA Copies)						
		Pre-challenge				Post-challenge		
		Day -2	Day 7	Day 14	Day 20	Day 28	Day 35	Day 42
T01	PCV2b	0	0	0	0	30421	280798	5184
T02	PCV2b	0	0	0	0	1866	22	2
T03	PCV2b	0	0	0	0	3870	238	10
T04	PCV2b	0	0	0	0	615	66	6
T05	PCV2a	0	0	0	0	10927	22982	1906
T06	PCV2a	0	0	0	0	43	2	2
T07	PCV2a	0	0	0	0	25991	211465	189
T08	PCV2a	0	0	0	0	309	20	6
T09	PCV2b-Divergent	0	0	0	0	413	384933	50683
T10	PCV2b-Divergent	0	0	0	0	7	314	65
T11	PCV2b-Divergent	0	0	0	0	406	427073	40711
T12	PCV2b-Divergent	0	0	0	0	189	3315	9

Table 5 PCV2 fecal shedding

Trt	Challenge	Time Point (Geometric LS Mean DNA Copies)			
		Pre-challenge	Post-challenge		
		Day 20	Day 28	Day 35	Day 42
T01	PCV2b	0	257	24822	106798
T02	PCV2b	0	7	7	0
T03	PCV2b	0	450	140	111
T04	PCV2b	0	43	288	16
T05	PCV2a	0	184	4130	23485
T06	PCV2a	0	0	16	2
T07	PCV2a	0	866	10072	6477
T08	PCV2a	0	387	73	20
T09	PCV2b-Divergent	0	1007	199324	89204
T10	PCV2b-Divergent	0	881	1880	65
T11	PCV2b-Divergent	0	58519	480005	219127
T12	PCV2b-Divergent	0	65	541	541

Pre-challenge titers, as measured by PCV2 ELISA, indicated that the least square (LS) mean titers of all treatment groups were PCV2 antibody negative (Table 6). PCV2 ELISA antibody titers >0.5 are considered to be PCV2 antibody positive.

Table 6 PCV2 ELISA

Trt	Challenge	Day -2	Day 7	Day 14	Day 20	Day 28	Day 35	Day 42
T01	PCV2b	0.1673	0.2741	0.1245	0.0241	0.0223	0.0995	0.3663
T02	PCV2b	0.1828	0.2934	0.1640	0.0644	0.3926	0.6212	0.5712
T03	PCV2b	0.1628	0.2783	0.1700	0.0183	0.0434	0.1833	0.2481
T04	PCV2b	0.1673	0.2593	0.1088	0.0278	0.1635	0.3956	0.3491
T05	PCV2a	0.2316	0.4322	0.2580	0.0320	0.0256	0.2396	0.3511
T06	PCV2a	0.1970	0.3726	0.2357	0.0887	0.4316	0.5458	0.4569
T07	PCV2a	0.2582	0.3787	0.2079	0.0399	0.0914	0.3066	0.4831
T08	PCV2a	0.1940	0.3437	0.2012	0.0357	0.2637	0.4717	0.4526
T09	PCV2b-Divergent	0.1334	0.2283	0.1603	0.0070	0.0102	0.0989	0.2374
T10	PCV2b-Divergent	0.1593	0.2071	0.1400	0.0518	0.4015	0.6827	0.6433
T11	PCV2b-Divergent	0.1752	0.2484	0.1340	0.0252	0.0807	0.2255	0.3105
T12	PCV2b-Divergent	0.1652	0.3035	0.1669	0.0270	0.2632	0.4868	0.4604

The experimental PCV2b divergent vaccine treatment (T04, T08, and T12) numerically reduced PCV2 viremia (Table 4) and fecal shed (Table 5). It also led to a decrease in histopathological lesions at most of the time points when compared to placebo, as demonstrated by immunohistochemistry (IHC) scores (Table 7), and lymphoid depletion scores (Table 8). Following challenge, a moderate anamnestic response in PCV2 ELISA antibody titers was observed in all challenge groups (Table 6), potentially suggesting that vaccine antigen dose needed further optimization. Although statistical comparisons were not made in this study, it is evident that the PCV2b divergent vaccine treatment afforded protection against the PCV2a, PCV2b, and PCV2b-Divergent challenge strains.

In assessing PCV2 vaccine efficacy, viremia and lymphoid depletion are considered by many to be the key parameters to measure. In this study, it is important to note that the PCV2b divergent vaccine performed numerically better against PCV2b divergent challenge than did either the PCV2a or PCV2b vaccines.

Table 7 Immunohistochemistry (IHC) scores

Trt	Challenge	Ever Abnormal?				Total Observations
		No		Yes		
		#	%	#	%	#
T01	PCV2b	2	22.2	7	77.8	9
T02	PCV2b	6	66.7	3	33.3	9
T03	PCV2b	4	50.0	4	50.0	8
T04	PCV2b	8	80.0	2	20.0	10
T05	PCV2a	3	30.0	7	70.0	10
T06	PCV2a	6	60.0	4	40.0	10
T07	PCV2a	4	44.4	5	5506	9
T08	PCV2a	7	70.0	3	30.0	10
T09	PCV2b-Divergent	4	40.0	6	60.0	10
T10	PCV2b-Divergent	9	100.0	0	0.0	9
T11	PCV2b-Divergent	2	22.2	7	77.8	9
T12	PCV2b-Divergent	7	77.8	2	22.2	9

Table 8 Lymphoid depletion scores

Trt	Challenge	Ever Abnormal?				Total Observations
		No		Yes		
		#	%	#	%	#
T01	PCV2b	3	33.3	6	66.7	9
T02	PCV2b	7	77.82	2	22.2	9
T03	PCV2b	7	87.5	1	12.5	8
T04	PCV2b	8	80.0	2	20.0	10
T05	PCV2a	4	40.0	6	60.0	10
T06	PCV2a	10	100.0	0	0	10
T07	PCV2a	6	66.7	3	33.3	9
T08	PCV2a	7	70.0	3	30.0	9
T09	PCV2b-Divergent	5	50.0	5	50.0	10
T10	PCV2b-Divergent	7	77.8	2	22.2	9
T11	PCV2b-Divergent	5	55.6	4	44.4	9
T12	PCV2b-Divergent	8	88.9	1	11.1	9

Example 5: PCV2b Challenge Model Optimization Study

The objective of this study was to assess PCV2b challenge material titrations, and route of administration. In addition, a preliminary assessment of a new PCV2b divergent challenge preparation was conducted alongside current validated PCV2a and PCV2b challenge models. An outline of the study design is shown in Table 9.

Table 9 PCV2b Challenge Model Optimization Study Design

Group	N	Challenge			Necropsy
		Strain	Titration	Dose, Route	
T01	12	PCV2b	4.7 log/3 mL	1 mL/ IM 2 mL/ IN	Necropsy (~9 wks of age) and Tissue Collection
T02	12		5.4 log/3 mL	1 mL/ IM 2 mL/ IN	
T03	12		6.1 log/3 mL	1 mL/ IM 2 mL/ IN	
T04	12		5.4 log/3 mL	3 mL/ IN	
T05	12	PCV2a	4.45 log/3 mL	1 mL/ IM 2 mL/ IN	
T06	12		5.15 log/3 mL	1 mL/ IM 2 mL/ IN	
T07	12		5.85 log/3 mL	1 mL/ IM 2 mL/ IN	
T08	12		5.15 log/3 mL	3 mL/ IN	
T09	12	PCV2b-Divergent	4.38 log/3 mL	1 mL/ IM 2 mL/ IN	
T10	12		5.08 log/3 mL	1 mL/ IM 2 mL/ IN	
T11	12		5.78 log/3 mL	1 mL/ IM 2 mL/ IN	
T12	12		5.08 log/3 mL	3 mL/ IN	

Crossbred pigs, approximately 6 weeks of age at Day 0, with low to negative serum antibody to PCV2, and PCV2 viremia-free, were placed in assigned pens/rooms in a BSL-2 facility with separate air spaces. There were 4 pens in each of the 3 rooms, with 12 pigs per pen. Pigs remained in the assigned pens throughout the study. Pigs had *ad libitum* access to water, and a non-medicated age-appropriate complete ration throughout the study. All pigs were allowed to acclimate for a minimum of 3 days.

Challenge was conducted on Day 0, when the pigs are about 6 weeks of age. Each pig was inoculated with a total of 3 mL of respective challenge virus, pre-diluted to 4.0-6.0 +/- 0.5 log10 TCID50 /mL, with 2 mL administered intranasally (IN) in each nostril and 1 mL intramuscularly (IM), or 3 mL IN, depending on the treatment group. A reserved aliquot of the challenge viruses was titrated following the challenge to confirm the actual challenge dose.

Individual blood samples (5-10 mL) were collected in serum separator tubes (SST) at Day -21, Day -1 (prior to vaccination), and Days 7, 14, and 21. Samples were aliquoted and stored at ≤ -65°C. Serum of Days -21, -1, 7, 14 and 21 was tested for PCV2 antibody titers by ELISA, and PCV2 viremia by qPCR.

Individual fecal swabs were taken from each pig prior to challenge (Day -1), and weekly post-challenge. Individual sterile polyester swabs were used for collecting fecal swabs, and placed in a tube containing 3 mL sterile PBS medium. Swabs were swirled for 5 seconds in the medium before being discarded. Samples were aliquoted and stored at ≤ -65°C. The fecal swab samples were tested for virus shedding by standard quantitative PCR procedures.

During necropsy, sections of tracheobronchial, mesentery and superficial inguinal lymph nodes, and tonsil tissues were collected in duplicate for each pig, individually identified, and fixed in 10% buffered formalin. One set was submitted for standard histopathology examination for lymphoid depletion (PCVAD), and histiocytic replacement. The conclusion was recorded as Yes (+) or No (-). A pig was considered having lymphoid depletion or histiocytic replacement if one or more tissues were scored "+". In addition, the tissues were also tested for PCV2 antigen by IHC. The results were recorded as 0 (no staining) and 1-3 (different levels of staining). A score of 0 was considered as PCV2 IHC (-), and a score of 1 or higher was considered as PCV2 IHC (+). A pig was considered IHC (+) if one or more tissues were IHC (+).

Due to the actual complexity of PCV epidemiology and the sensitivity of PCV2 qPCR, it is possible that some pigs may become viremic prior to challenge. Pigs that test viremic prior to challenge may be removed from the study, and may be excluded in the data analysis based on the discretion of the clinical sponsor.

The primary outcomes are the PCV2b divergent challenge isolate tested in comparison to the validated models for PCV2a and PCV2b.

Results

Animals administered the PCV2b Divergent challenge isolate had an increase in antibody titers from prior to challenge to the end of study across treatment groups. The undiluted challenge group followed by the T12 group (Diluted 1:5, administered IN) had the peak viremia at 14 days after challenge, with over 4 million and 1 million DNA copies/mL, respectively. The peak PCV2 shedding of the undiluted challenge material was 754,114 DNA copies/mL. The undiluted IM/IN and 1:5 IN only, resulted in the highest number of animals positive for histopathological abnormalties and PCV2 colonization.

Based on data collected from this PCV2b challenge optimization study (data not shown), challenge route and dose changed to 3 mL intranasal. The change in challenge route and dose is thought to decrease the chances of an adverse event thought to be caused by intramuscular administration and increase overall challenge take.

Example 6: Evaluation of Two Vaccine Candidates Against a PCV2b Challenge

The objective of the study was to assess the protection of a chimeric PCV2b vaccine and a PCV2b divergent vaccine, each represented at a low and high antigen dose, against a PCV2b challenge. The study design is outlined in Table 10. The placebo (T01) was 10% SP-Oil. The IVP's were as follows: T02, killed PCV1:PCV2b capsid chimera low dose (cPCV2b low), adjuvanted with 10% SP-Oil; T03, killed PCV1:PCV2b capsid chimera high dose (cPCV2b high), adjuvanted with 10% SP-Oil; T04, killed PCV2b-divergent vaccine low dose (PCV2b DIV low), adjuvanted with 10% SP-Oil; T05, killed PCV2b-divergent vaccine high dose (PCV2b DIV high), adjuvanted with 10% SP-Oil. The vaccines were produced using 20x concentrated antigen and then formulating the vaccine at: 0.69% antigen input = low dose or 3.00% antigen input = high dose.

Table 10 Study Design

Group	N	Investigational Veterinary Product (IVP)	Vaccinations	Challenge			Necropsy
				Day	Strain	Dose, Route	
T01	12	Placebo	Day 0 (~3 wks of age) 2 mL IM Right side of neck	Day 21 (~6 wks of age)	PCV2b	3 mL IN	Day 42 (~9 wks of age) and Tissue Collection
T02	12	Killed, adjuvanted PCV1:PCV2b chimera (low dose)					
T03	12	Killed, adjuvanted PCV1:PCV2b chimera (high dose)					
T04	12	Killed, adjuvanted PCV2b-Divergent (low dose)					
T05	12	Killed, adjuvanted PCV2b-Divergent (high dose)					

Pigs were ~3 weeks of age (21 ±7 days of age) on Day 0 for vaccination. A treatment administrator administered a single dose of 2 mL of the assigned vaccine intramuscularly (IM) into the right side of the neck. A single 3 mL sterile syringe with 1” or ¾” needle was used for each pig. Vaccination details were recorded. Pigs were observed within 1 hour (± 30 minutes) after each vaccination for abnormal clinical signs, including but not limited to: lethargy, labored breathing, vomiting, and incoordination. Any observed clinical signs were documented on the general health form. A veterinarian was notified to follow up on the pig(s) with any of the signs described above.

Challenge was conducted on Day 21 when the pigs were about 6 weeks of age. Each pig was inoculated with a total 3 mL intranasally (IN) of a culture of a virulent PCV2b strain, pre-diluted to 4.8-5.8 log10 TCID50 /mL. A reserved aliquot of the challenge viruses was titrated following the challenge to confirm the actual challenge dose.

Individual blood samples (5-10 mL) were collected in serum separator tubes (SST) on Day -1 (prior to vaccination), and Days 7, 14, 20/21, 28, 35, and 42. Samples were aliquoted and stored at ≤ -65°C. They were later tested for PCV2 antibody titers by ELISA and PCV2 viremia by qPCR.

Individual fecal swabs were taken from each pig prior to challenge (Day 20/21), and weekly post-challenge. Individual sterile polyester swabs were used for collecting fecal swab and placed in a tube containing 3 mL sterile PBS medium. Swabs were swirled for 5 seconds in the medium before discarded. Samples were aliquoted and stored at ≤ -65°C. The fecal swab samples were tested for virus shedding by standard quantitative PCR procedure.

During necropsy, sections of tracheobronchial, mesentery and superficial inguinal lymph nodes, and tonsil tissues were collected in duplicate for each pig, individually identified and fixed in 10% buffered formalin. One set was archived, while the other was submitted for standard histopathology examination for lymphoid depletion (PCVAD), and histiocytic replacement. The conclusion was recorded as Yes (+) or No (-). A pig was considered having lymphoid depletion or histiocytic replacement if one or more tissues were scored "+". In addition, the tissues were also tested for PCV2 antigen by IHC. The results were recorded as 0 (no staining) and 1-3 (different levels of staining). A score 0 was considered as PCV2 IHC (-), and a score of 1 or higher was considered as PCV2 IHC (+). A pig was considered IHC (+) if one or more tissues are IHC (+).

The primary outcome was the protection of one of the four candidate vaccines against the PCV2b challenge, when compared to the placebo. The primary variable was viremia, and the secondary variables were fecal shed and histopathological lesions.

Results

PCV2 Viremia

Serum was collected weekly and analyzed for PCV2 viremia by quantitative PCR. Geometric least square means of each study day are illustrated in Figure 2. All pigs stayed negative for PCV2 viremia prior to challenge, as demonstrated in Table 11 below.

Table 11 PCV2 Viremia (DNA Copies) as Tested by qPCR by Study Day

Trt	Serial	Time Point (Geometric LS Mean DNA Copies)						
		Pre-challenge				Post-challenge		
		Day -1	Day 7	Day 14	Day 20	Day 28	Day 35	Day 44
T01	Placebo	0	0	0	0	30	132010.6	24113.9
T02	cPCV2b low	0	0	0	0	3.8	0	0
T03	cPCV2b high	0	0	0	0	1.2	4.5	0
T04	PCV2b DIV low	0	0	0	0	29.9	12.1	1.4
T05	PCV2b DIV high	0	0	0	0	1.2	0	0

PCV2 Viremia (DNA Copies) by treatment and challenge are described below in Table 12. All treatment groups were significantly different from the T01 group post challenge on days 35 and 44 (P<0.0001).

Percent of animals that were ever positive throughout the course of the study are listed below (Table 12). The placebo group had a significantly higher number of animals that were ever positive compared to the vaccinated groups (P<0.0124).

Table 12 qPCR Qualitative Serum Viremia - Percent Ever Positive

Serial	Ever Positive?				Total Observations	P-Value
	Pos		Neg			
	#	%	#	%	Number	
Placebo	11	100.0	0	0	11	
cPCV2b low	2	16.7	10	83.3	12	0.0001
cPCV2b high	2	16.7	10	83.3	12	0.0001
PCV2b DIV low	5	45.5	6	54.5	11	0.0124
PCV2b DIV high	1	8.3	11	91.7	12	0.0001

PCV2 Fecal Shedding

Fecal shedding geometric least square means by study day are illustrated in Figure 3. PCV2 fecal shedding (DNA Copies) by treatment and challenge are described below in Table 13. All treatment groups were significantly different from the T01 group post challenge on days 35 and 44 (P<0.0001).

Table 13 PCV2 Fecal Shed (DNA Copies) as Tested by qPCR

Trt	Serial	Time Point (Geometric LS Mean DNA Copies)			
		Prior to Challenge	Post-challenge		
		Day 20	Day 28	Day 35	Day 44
T01	Placebo	0	29.9	65713.8	19996.2
T02	cPCV2b low	0	3.8	3.8	0
T03	cPCV2b high	0	1.2	1.3	0
T04	PCV2b DIV low	0	29.9	12.1	1.4
T05	PCV2b DIV high	0	1.2	9.6	0

Percent of animals that were ever positive for shedding throughout the course of the study are listed below (Table 14). The placebo group had a significantly higher number of animals that were ever shedding compared to the vaccinated groups (P<0.0124).

Table 14 qPCR Qualitative Fecal shedding - Percent Ever Positive

Serial	Ever Positive?				Total Observa-tions	P-Value
	Pos		Neg			
	#	%	#	%	Number	
Placebo	11	100.0	0	0	11	
cPCV2b low	3	25.0	9	75.0	12	0.0003
cPCV2b high	1	8.3	11	91.7	12	≤ 0.0001
PCV2b DIV low	5	45.5	6	54.5	11	0.0124
PCV2b DIV high	4	33.3	8	66.7	12	0.0013

Serum Antibody Response

PCV2 antibody titer means of each treatment by study day are illustrated in Figure 4. All pigs were PCV2 seronegative prior to vaccination. Pigs in the Placebo group remained seronegative prior to challenge.

PCV2 ELISA antibody titers are summarized in Table 15 below. All titers >0.5 are considered to be PCV2 antibody positive. Pigs in all vaccine groups showed significant increases ($P \leq 0.0895$) of PCV2 antibody titer on Days 28-44 post vaccination when compared to placebo, indicating the active immune response to PCV2 following vaccination. In addition, the T03 and T05 also had significantly higher titers on day 20 post vaccination ($P \leq 0.0684$ and $P \leq 0.0738$, respectively).

Table 15 PCV2 ELISA S/P LS Mean Titers by Study Day

Treatment	Day -1	Day 7	Day 14	Day 20	Day 28
Placebo	0.215	0.172	0.092	0.066	0.046
cPCV2b low	0.203	0.168	0.119	0.113	0.266*
cPCV2b high	0.195	0.156	0.118	0.138*	0.214*
PCV2b DIV low	0.193	0.176	0.090	0.086	0.172*
PCV2b DIV high	0.233	0.191	0.125	0.137*	0.157*
Treatment	**Day 35**	**Day 44**			
Placebo	0.225	0.526			
cPCV2b low	0.715*	0.807*			
cPCV2b high	0.667*	0.706*			
PCV2b DIV low	0.563*	0.685*			
PCV2b DIV high	0.702*	0.783*			

Histopathology: Lymphoid Depletion (LD) and Virus Infection in Lymphoid Tissues (IHC)

PCV2 percent abnormal histopathology scores (data not shown) did not demonstrate a significant difference between the placebo and vaccinated groups when considering lymphoid lesions and the presence of PCV2 antigens.

The data from this study indicated that all pigs on study up to the challenge on Day 21 remained free of PCV2 infection as evidenced by 1) lack of detectable PCV2 DNA in serum collected at weekly intervals from the time of vaccination to the time of challenge and 2) Lack of serologic evidence among the T01 group that there was any unintended exposure to PCV2 prior to challenge. All vaccines significantly protected vaccinated animals from becoming viremic post challenge. Also, all vaccines significantly reduced fecal shedding of PCV2 post challenge in vaccinated animals. Pigs in all vaccine groups showed significant increases ($P \leq 0.0895$) of PCV2 antibody titer on Days 28-44 post vaccination when compared to placebo, indicating an active immune response to PCV2 following vaccination. There was a numerical reduction in colonization (IHC) in all vaccinated groups versus controls, but it was not statistically significant. The lack of significant difference between the groups could have been due to the weak challenge take seen in the control group.

Example 7: Evaluation of Two Vaccine Candidates Against a PCV2b-divergent Challenge

The objective of the study was to assess the protection of a chimeric PCV2b vaccine and a PCV2b divergent vaccine, each represented at a low and high antigen dose, as well as a PCV2a capsid expressed in baculovirus, against a PCV2b divergent challenge. The study design is outlined in Table 16. The placebo (T01) was 10% SP-Oil. The IVP's were as follows: T02, killed PCV1:PCV2b capsid chimera low dose (cPCV2b low), adjuvanted with 10% SP-Oil; T03, killed PCV1:PCV2b capsid chimera high dose (cPCV2b high), adjuvanted with 10% SP-Oil; T04, killed PCV2b-divergent vaccine low dose (PCV2b DIV low), adjuvanted with 10% SP-Oil; T05, killed PCV2b-divergent vaccine high dose (PCV2b DIV high), adjuvanted with 10% SP-Oil; T06, killed baculovirus expressing a PCV2a capsid, in an aqueous-based adjuvant (comparative product). The T02-T05 vaccines were produced using 20x concentrated antigen and then formulating the vaccine at: 0.69% antigen input = low dose or 3.00% antigen input = high dose.

Table 16 Study Design

Group	N	Investigational Veterinary Product (IVP)	Vaccinations	Challenge			Necropsy
				Day	Strain	Dose, Route*	
T01	12	Placebo	Day 0 (~3 wks of age) 2 mL IM Right side of neck	Day 21 (~6 wks of age)	PCV2b-divergent	3 mL IN	Day 42 (~9 wks of age) and Tissue Collection
T02	12	Killed, adjuvanted PCV1:PCV2b chimera (low dose)					
T03	12	Killed, adjuvanted PCV1:PCV2b chimera (high dose)					
T04	12	Killed, adjuvanted PCV2b-Divergent (low dose)					
T05	12	Killed, adjuvanted PCV2b-Divergent (high dose)					
T06	12	Killed baculovirus expressing PCV2a capsid	1 mL IM Right side of neck				

Pigs were ~3 weeks of age (21 ± 8 days of age) on Day 0 for vaccination. A treatment administrator administered a single dose of 2 mL (T01-T05) or 1 ml (T06) of the assigned vaccine intramuscularly (IM) into the right side of the neck. A single 3 mL sterile syringe with 1” or ¾” needle was used for each pig. Vaccination details were recorded. Pigs were observed within 1 hour (± 30 minutes) after each vaccination for abnormal clinical signs, including but not limited to: lethargy, labored breathing, vomiting, and incoordination. Any observed clinical signs were documented on the general health form. A veterinarian was notified to follow up on the pig(s) with any of the signs described above.

Challenge was conducted on Day 21 when the pigs were about 6 weeks of age. Each pig was inoculated with a total 3 mL intransally (IN) of a culture of a virulent PCV2b-divergent strain, pre-diluted to 4.8-5.8 log10 TCID50 /mL. A reserved aliquot of the challenge viruses was titrated following the challenge to confirm the actual challenge dose.

Individual blood samples (5-10 mL) were collected in serum separator tubes (SST) on Day -1 (prior to vaccination), and Days 7, 14, 20/21, 28, 35, and 42. Samples were aliquoted and stored at ≤ -65°C. They were later tested for PCV2 antibody titers by ELISA and PCV2 viremia by qPCR.

Individual fecal swabs were taken from each pig prior to challenge (Day 20/21), and weekly post-challenge. Individual sterile polyester swabs were used for collecting fecal swab and placed in a tube containing 3 mL sterile PBS medium. Swabs were swirled for 5 seconds in the medium before discarded. Samples were aliquoted and stored at ≤ -65°C. The fecal swab samples were tested for virus shedding by standard quantitative PCR procedure.

During necropsy, sections of tracheobronchial, mesentery and superficial inguinal lymph nodes, and tonsil tissues were collected in duplicate for each pig, individually identified and fixed in 10% buffered formalin. One set was archived, while the other was submitted for standard histopathology examination for lymphoid depletion (PCVAD), and histiocytic replacement. The conclusion was recorded as Yes (+) or No (-). A pig was considered having lymphoid depletion or histiocytic replacement if one or more tissues were scored "+". In addition, the tissues were also tested for PCV2 antigen by IHC. The results were recorded as 0 (no staining) and 1-3 (different levels of staining). A score 0 was considered as PCV2 IHC (-), and a score of 1 or higher was considered as PCV2 IHC (+). A pig was considered IHC (+) if one or more tissues were IHC (+).

The primary outcome was the protection of one of four candidate vaccines and the baculovirus vaccine against the PCV2b-divergent challenge, when compared to the placebo. The primary variable was viremia, and the secondary variables were fecal shed and histopathological lesions.

PCV2 Viremia

Serum was collected weekly and analyzed for PCV2 viremia by quantitative PCR. Geometric least square means of each study day are illustrated in Figure 5. All pigs except one animal in both the T04 and T05 groups stayed negative for PCV2 viremia prior to challenge, as demonstrated in Table 17 below.

Table 17 PCV2 Viremia (DNA Copies) as Tested by qPCR by Study Day

Trt	Serial	Time Point (LS Mean DNA Copies)						
		Pre-challenge				Post-challenge		
		Day -1	Day 7	Day 14	Day 20	Day 28	Day 35	Day 44
T01	Placebo	0	0	0	0	12.2	91196.2	22165.7
T02	cPCV2b low	0	0	0	0	3.8	68	4.9
T03	cPCV2b high	0	0	0	0	0.0	1.2	0.0
T04	PCV2b DIV low	0	0	0	1.2	1.5	3.8	0.0
T05	PCV2b DIV high	0	0	0	1.2	0.0	3.8	0.0
T06	baculovirus expressing PCV2a capsid	0	0	0	0.0	1.2	86.0	3.8

Percent of animals that were ever positive throughout the course of the study are listed below (Table 18). The placebo group had a significantly higher percentage of animals that were ever positive compared to the vaccinated groups ($P \leq 0.0046$).

Table 18 qPCR Qualitative Serum Viremia - Percent Ever Positive

Trt	Serial	Ever Positive?				Total Observations	P-Value
		Pos		Neg			
		#	%	#	%	Number	
T01	Placebo	12	100.0	0	0	12	
T02	cPCV2b low	5	41.7	7	58.3	12	0.0046
T03	cPCV2b high	1	8.3	11	91.7	12	0.0001
T04	PCV2b DIV low	2	16.7	10	83.3	12	0.0001
T05	PCV2b DIV high	2	16.7	10	83.3	12	0.0001
T06	baculovirus expressing PCV2a capsid	5	41.7	7	58.3	12	0.0046

PCV2 Fecal Shedding

Fecal shedding geometric least square means by study day are illustrated in Figure 6. PCV2 fecal shedding (DNA Copies) by treatment and challenge are described below in

Table 19. One animal in the T04 group was shedding the day prior to challenge. All vaccine groups were shedding significantly lower ($P \leq 0.0001$) least squares mean PCV2 DNA copy numbers than the placebo group on Days 35 and 43 post challenge. In addition, the T03, T05 and T06 groups were also noted with shedding significantly lower least squares mean DNA copies ($P \leq 0.0830$) on Day 28 of study.

Table 19 PCV2 Fecal Shed (DNA Copies) as Tested by qPCR

Trt	Serial	Time Point (Geometric LS Mean DNA Copies)			
		Prior to Challenge	Post-challenge		
		Day 20	Day 28	Day 35	Day 44
T01	Placebo	0	22.3	24228.5	10281.5
T02	cPCV2b low	0	111.3	36.2	5.1
T03	cPCV2b high	0	1.2	0.0	0.0
T04	PCV2b DIV low	1.2	1.7	1.2	5.0
T05	PCV2b DIV high	0	0.0	0.0	0.0
T06	baculovirus expressing PCV2a capsid	0	0.0	3.8	0.0

The percent of animals that were ever positive for shedding throughout the course of the study are listed below (Table 20). Following challenge, when compared to the placebo group, groups T03-T06 had a significant reduction ($P \leq 0.0028$) in the percent of pigs shedding PCR detectable PCV2 DNA.

Table 20 qPCR Qualitative Fecal shedding - Percent Ever Positive

	Ever Positive?				Total Observa-tions	
	Pos		Neg			
	#	%	#	%	Number	P-Value
Placebo	11	91.7	1	8.3	12	
cPCV2b low	8	66.7	4	33.3	12	0.3168
cPCV2b high	1	8.3	11	91.7	12	0.0001
PCV2b DIV low	3	25.0	9	75.0	12	0.0028
PCV2b DIV high	0	0	12	100.0	12	0.0001
baculovirus expressing PCV2a capsid	2	16.7	10	83.3	12	0.0006

Serum Antibody Response

With respect to PCV2 antibody titers, the results indicated that the PCV2b divergent vaccine treatments (T04; T05) had a stronger serologic response compared to the other treatments prior to challenge at Study Day 21, as assessed by ELISA (Table 21; Figure 7). Following challenge, however, the PCV2b divergent treatments did not respond as strongly as the other treatments (Figure 7). One possible conclusion is that the animal already had a specific strong anti-PCV2b divergent antibody response, and was able to neutralize and eliminate the challenge virus very quickly. This translated to a decreased antibody response post-challenge, when compared to that of the heterologous vaccines. While serology is not the same as efficacy, it has been demonstrated that declining antibody titers in pigs receiving an efficacious vaccine indicates protection (Thacker et al., 2013, Proc AASV, 217).

Table 21 PCV2 ELISA

Treatment	Study Day						
	-1	7	14	20	28	35	43
T01	0.2094	0.1427	0.0937	0.0824	0.0539	0.0877	0.3064
T02	0.1943	0.1249	0.1133	0.0981	0.1328	0.7637*	0.7935*
T03	0.2102	0.1377	0.1458	0.1671*	0.2362*	0.8084*	0.7973*
T04	0.1856	0.1399	0.1467	0.1560	0.2388*	0.4836*	0.6209*
T05	0.2064	0.1274	0.0772	0.1362	0.2902*	0.5438*	0.5749*
T06	0.1814	0.1283	0.0999	0.1089	0.1627	0.8350*	0.8902*

***P-Value<0.10 vs. T01**

Histopathology: Lymphoid Depletion (LD), and Virus Infection in Lymphoid Tissues (IHC)

At the time of necropsy, when compared to the placebo group, all vaccine groups had significantly less percentage of animals with microscopic lymphoid lesions (LD) and PCV2 antigen colonization (IHC), $P \leq 0.0995$.

The PCV2 IHC data are summarized in Table 22 below.

Table 22 PCV2 IHC Scores: If lymphoid or tonsil tissues ever abnormal

Trt	Serial	Ever Abnormal?				Total Obs
		No		Yes		
		#	%	#	%	Number
T01	Placebo	4	33.3	8	66.7	12
T02	cPCV2b low	9	75	3	25	12
T03	cPCV2b high	10	83.3	2	16.7	12
T04	PCV2b DIV low	11	91.7	1	8.3	12
T05	PCV2b DIV high	12	100	0	0	12
T06	baculovirus expressing PCV2a capsid	12	100	0	0	12

The PCV2 Lymphoid Depletion (LD) data are summarized in Table 23 below.

Table 23 PCV2 Lymphoid Depletion Scores: If lymphoid or tonsil tissues ever abnormal

	Ever Abnormal?				Total Obs
	No		Yes		
Serial	#	%	#	%	Number
Placebo	4	33.3	8	66.7	12
cPCV2b low	9	75	3	25	12
cPCV2b high	12	100	0	0	12
PCV2b DIV low	10	83.3	2	16.7	12
PCV2b DIV high	11	91.7	1	8.3	12
baculovirus expressing PCV2a capsid	12	100	0	0	12

The data from this study indicated that_all treatment groups least squares mean PCV2 titers were seronegative prior to vaccination. Pigs in the Placebo group remained seronegative prior to challenge. One animal in both the T04 and T05 groups were viremic the day prior to challenge. The animal in the T04 group was also shedding, however less than 10% of the animals became viremic prior to challenge and the study was considered valid. Following challenge, when compared to the placebo group, all vaccinated groups had a significant reduction in the percent of viremic pigs. Following challenge, when compared to the placebo group, groups T03-T06 had a significant reduction in the percent of pigs shedding PCR detectable PCV2 DNA. At necropsy, when compared to the placebo group, all vaccine groups had significantly less percentage of animals with microscopic lymphoid lesions (LD) and PCV2 antigen colonization. The study demonstrated that the cPCV1-2b, PCV2b divergent and baculovirus expressing PCV2a capsid vaccines cross protect against a PCV2b divergent strain challenge.

It is to be understood that the examples above are provided by way of illustration only, and nothing therein should be deemed a limitation upon the overall scope of the invention.

What is Claimed is:

1. A vaccine composition for protecting pigs against PCV2, including a highly virulent porcine circovirus type 2b (PCV2b) divergent strain, the composition comprising a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide comprises Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134, according to the numbering of SEQ ID NO: 1.

2. The composition of claim 1, wherein the composition is in the form of an inactivated, PCV2b divergent whole virus that comprises and/or expresses the PCV2b divergent ORF2 polypeptide.

3. The composition of claim 1, wherein the composition is in the form of an inactivated chimeric virus, wherein said chimeric virus comprises an inactivated recombinant porcine circovirus type 1 that comprises and/or expresses the PCV2b divergent ORF2 polypeptide.

4. The composition of claim 1, wherein the composition is in the form of an isolated, recombinant PCV2b divergent ORF2 polypeptide.

5. The composition of claim 4, wherein the isolated, recombinant PCV2b divergent ORF2 polypeptide is expressed from a vector.

6. The composition of claim 5, wherein the vector is baculovirus or parapoxvirus.

7. The composition of claim 5, wherein the vector is a live or inactivated vector.

8. The composition of any one of claims 1 to 7, wherein the PCV2b divergent ORF2 polypeptide further comprises at least one residue selected from the group consisting of: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a

Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215 according to the numbering of SEQ ID NO: 1.

9. The composition of any one of claims 1 to 7, wherein the PCV2b divergent ORF2 polypeptide further comprises a Lysine (K) at residue 59 and a Lysine (K) at residue 234 according to the numbering of SEQ ID NO: 1.

10. The composition of claim 9, wherein the PCV2b divergent ORF2 polypeptide further comprises a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215 according to the numbering of SEQ ID NO: 1.

11. The composition of any one of claims 1 to 10, wherein the PCV2 divergent ORF2 polypeptide is represented by the amino acid sequence of SEQ ID NO: 1, or a fragment thereof.

12. The composition of any one of claims 1 to 11, further comprising at least one additional porcine antigen.

13. The composition of claim 12, wherein the at least one additional antigen is protective against a disease in pigs caused by a microorganism.

14. The composition of claim 13, wherein the microorganism comprises a bacterium, virus, or protozoan.

15. The composition of claim 14, wherein the microorganism is selected from the group consisting of *Mycoplasma hyopneumoniae* (M. hyo), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), *Haemophilus parasuis, Pasteurella multocida, Streptococcum suis, Staphylococcus hyicus, Actinobacilllus pleuropneumoniae, Bordetella*

bronchiseptica, Salmonella choleraesuis, Salmonella enteritidis, Erysipelothrix rhusiopathiae, *Mycoplama hyorhinis, Mycoplasma hyosynoviae*, leptospira bacteria, *Lawsonia intracellularis*, swine influenza virus (SIV), *Escherichia coli* antigen, *Brachyspira hyodysenteriae*, porcine respiratory coronavirus, Porcine Epidemic Diarrhea (PED) virus, rotavirus, Torque teno virus (TTV), Porcine Cytomegalovirus, Porcine enteroviruses, Encephalomyocarditis virus, a pathogen causative of Aujesky's Disease, Classical Swine fever (CSF) and a pathogen causative of Swine Transmissable Gastroenteritis, or combinations thereof.

16. The composition of any one of claims 1 to 15, wherein the composition further comprises an adjuvant.

17. The composition of claim 16, wherein the adjuvant is selected from the group consisting of an oil-in-water adjuvant, a polymer and water adjuvant, a water-in-oil adjuvant, an aluminum hydroxide adjuvant, a vitamin E adjuvant and combinations thereof.

18. The composition of any one of claims 1 to 17, wherein the composition further comprises a pharmaceutically acceptable carrier.

19. A method of immunizing a pig against a PCV2b divergent strain, the method comprising administering to the pig the composition of any one of claims 1 to 18.

20. The method of claim 19, wherein the composition is administered intranasally, intramuscularly, intradermally, transdermally, subcutaneously, or orally.

21. The method of claim 19 or claim 20, wherein the composition is administered in a single dose.

22. The method of claim 19 or claim 20, wherein the composition is administered as two doses.

23. The method of any one of claims 19 to 22, wherein the composition is administered to pigs having maternally-derived antibodies against PCV2.

24. The method of any one of claims 19 to 23, wherein the composition is administered to pigs at 3 weeks of age or older.

25. A kit comprising: a bottle comprising a vaccine composition for protecting pigs against a highly virulent porcine circovirus type 2b (PCV2b) divergent strain, the composition comprising a PCV2b divergent ORF2 polypeptide, wherein the ORF2 polypeptide comprises Leucine (L) at position 89, Threonine (T) at position 90, and Aspargine (N) at position 134 according to the numbering of SEQ ID NO: 1.

26. The kit of claim 25, wherein the PCV2b divergent ORF2 polypeptide further comprises at least one residue selected from the group consisting of: a Lysine (K) at residue 59, a Lysine (K) at residue 234, a Threonine (T) at residue 190, an Isoleucine (I) at residue 53, an Asparagine (N) at residue 68, an Arginine (R) or Glycine (G) at residue 169, and an Isoleucine (I) at residue 215 according to the numbering of SEQ ID NO: 1.

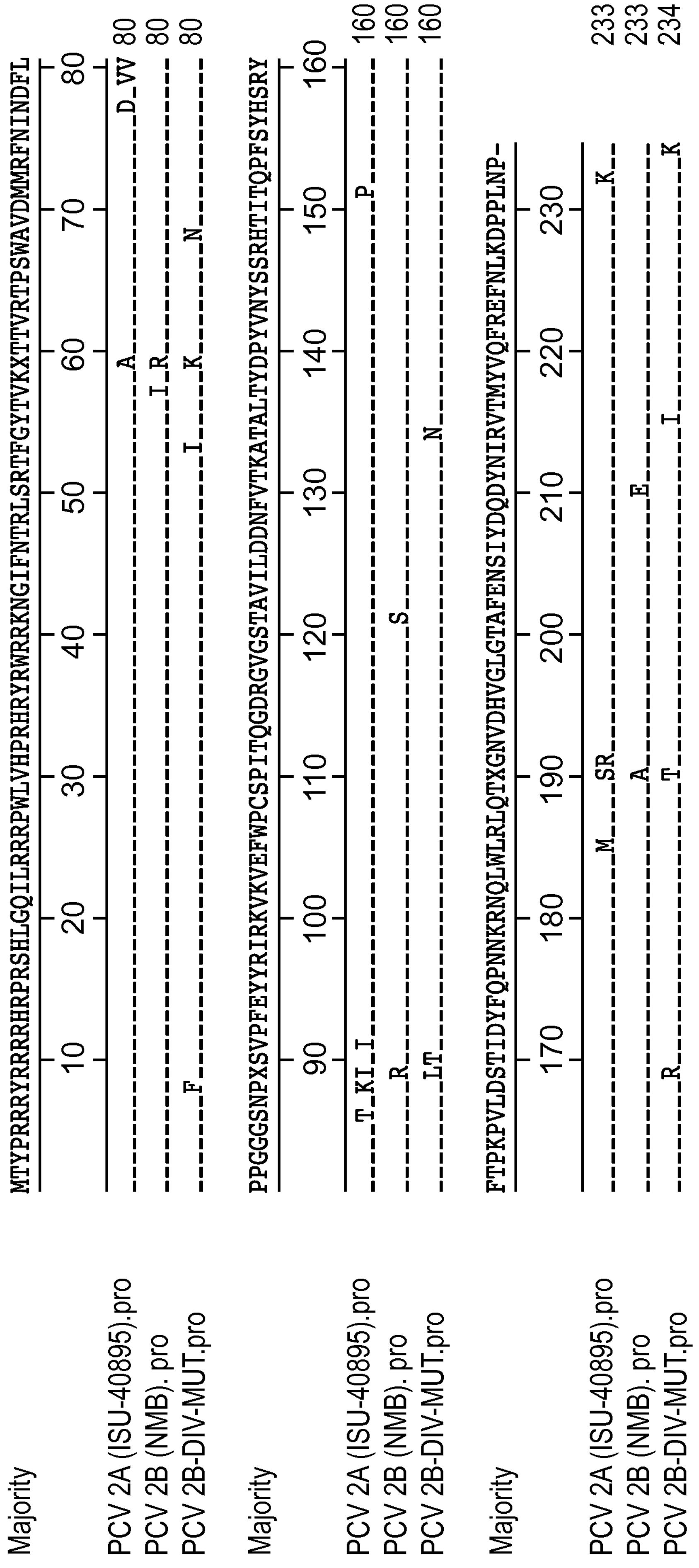
27. The kit of claim 25 or claim 26, wherein the composition in the bottle is provided as a ready-to-use liquid composition.

28. The kit of claim 25 or claim 26, wherein the composition is provided in a lyophilized form.

29. The kit of claim 28, further comprising a diluent.

30. The kit of any one of claims 25 to 29, further including an instruction manual which contains the information for administration of the vaccine composition.

FIG. 1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PCV 2A (ISU-40895).pro
PCV 2B (NMB). pro
PCV 2B-DIV-MUT.pro
Majority
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PCV 2A (ISU-40895).pro
PCV 2B (NMB). pro
PCV 2B-DIV-MUT.pro
Majority
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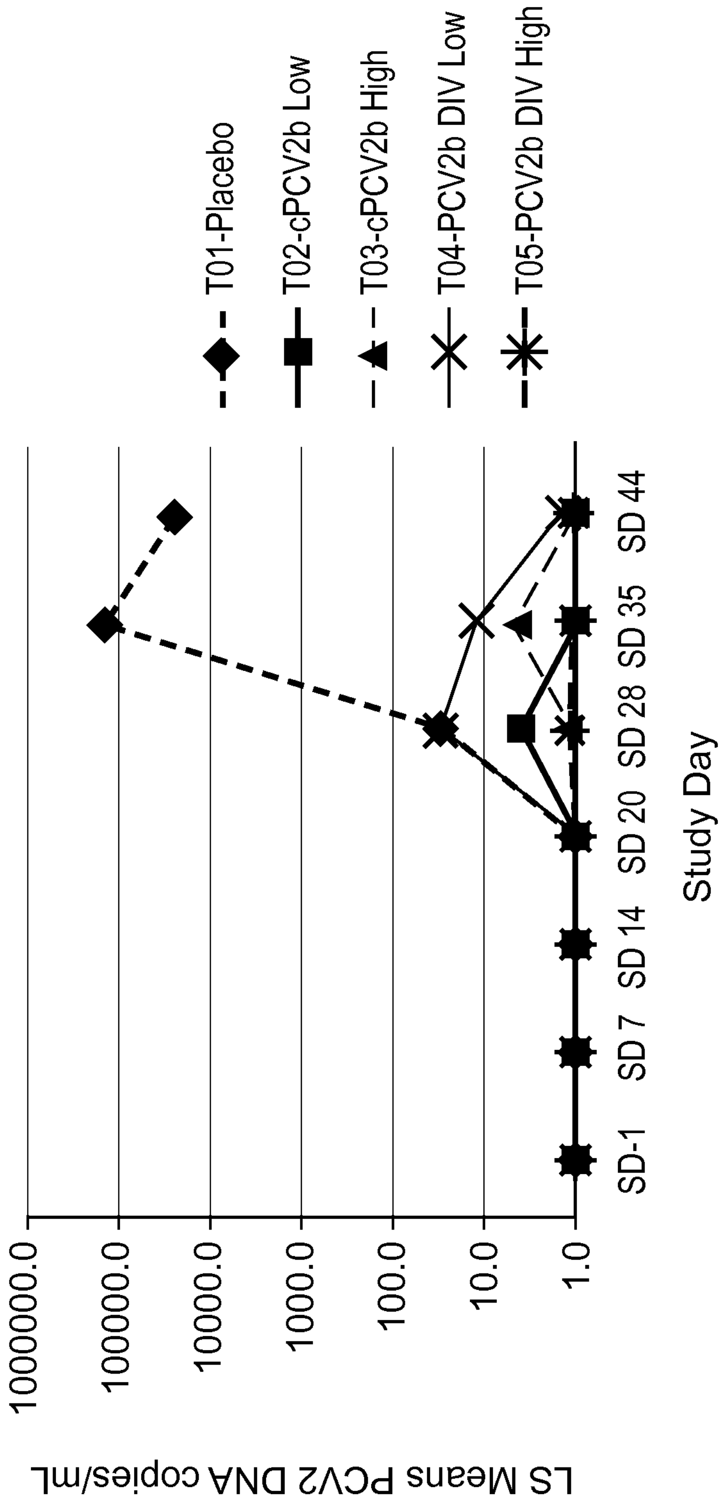
PCV 2A (ISU-40895).pro
PCV 2B (NMB). pro
PCV 2B-DIV-MUT.pro

FIG. 2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T02-cPCV2b Low
T03-cPCV2b High
T04-PCV2b DIV Low
T05-PCV2b DIV High
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100000.0
10000.0
1000.0
100.0
10.0
1.0
LS Means PCV2 DNA copies/mL
SD-1
SD 7
SD 14
SD 20
SD 28
SD 35
SD 44
Study Day

FIG. 3

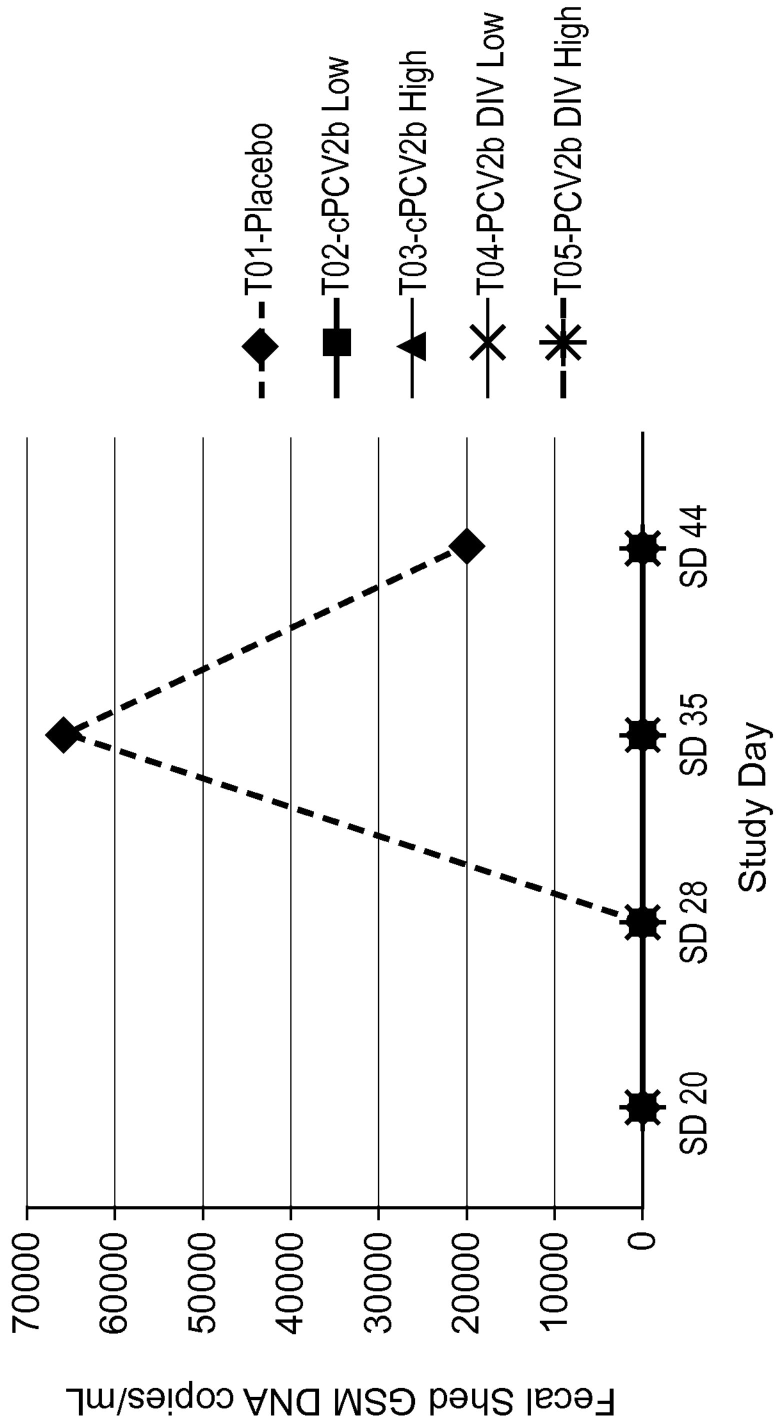

Fecal Shed GSM DNA copies/mL
70000
60000
50000
40000
30000
20000
10000
0
SD 20
SD 28
SD 35
SD 44
Study Day
T01-Placebo
T02-cPCV2b Low
T03-cPCV2b High
T04-PCV2b DIV Low
T05-PCV2b DIV High

FIG. 4

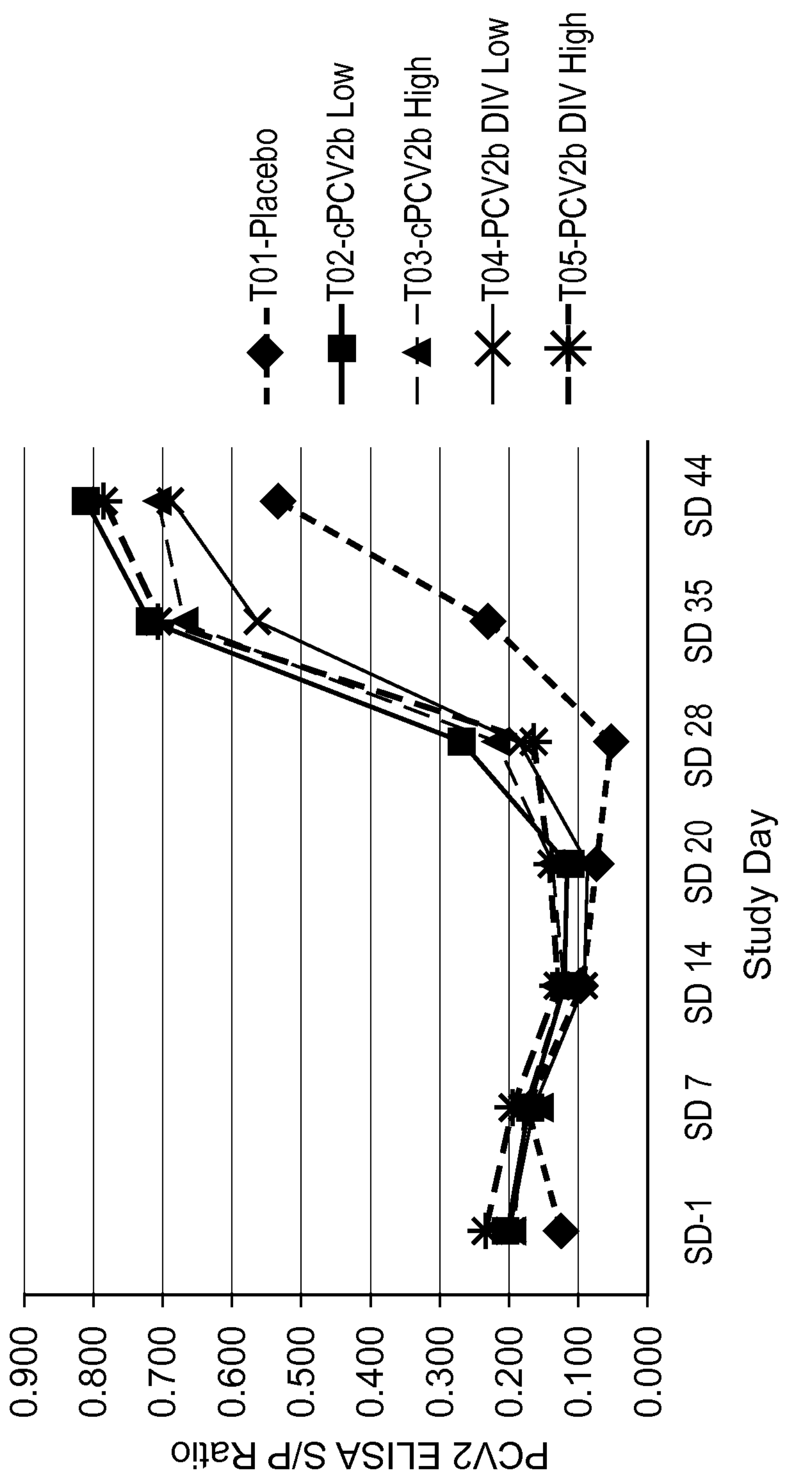

T01-Placebo
T02-cPCV2b Low
T03-cPCV2b High
T04-PCV2b DIV Low
T05-PCV2b DIV High
PCV2 ELISA S/P Ratio
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
SD-1
SD 7
SD 14
SD 20
SD 28
SD 35
SD 44
Study Day

FIG. 5

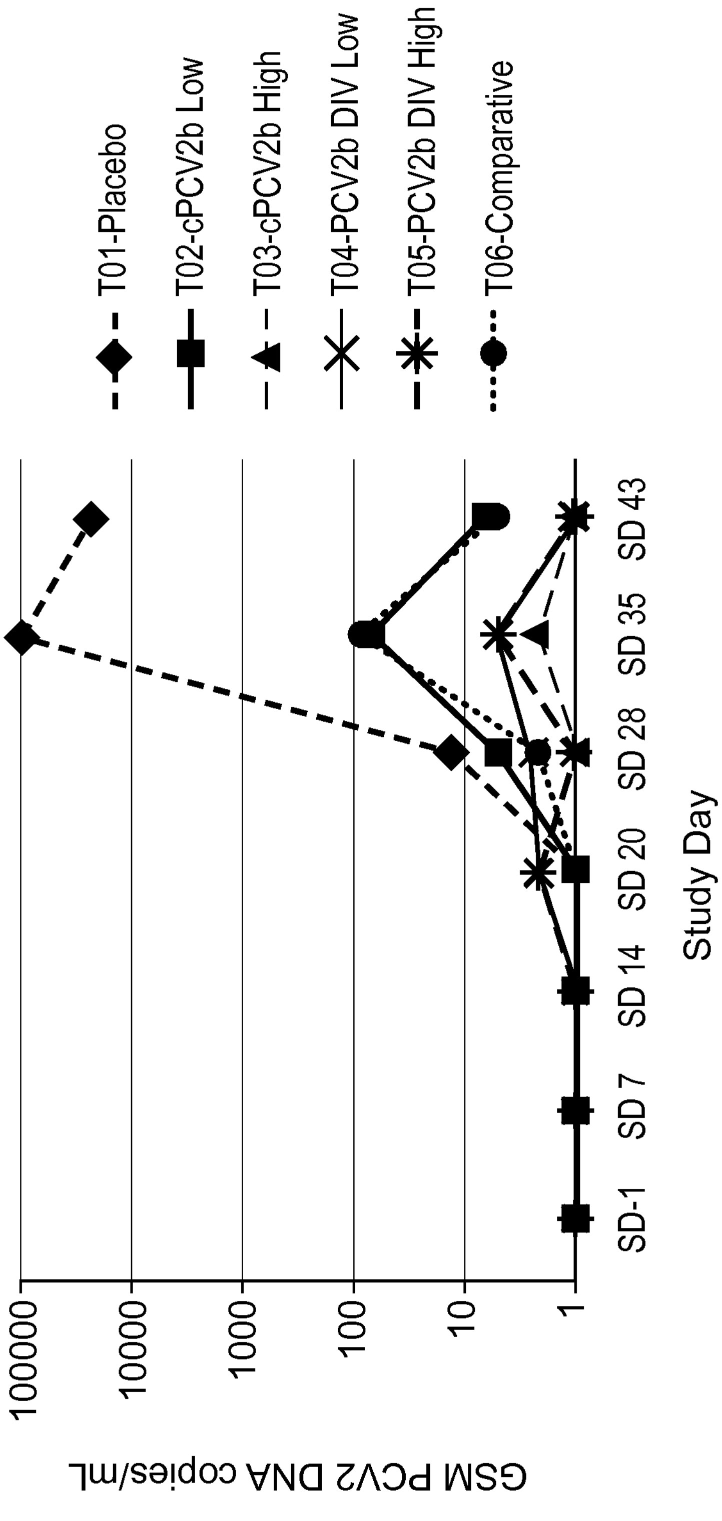

GSM PCV2 DNA copies/mL
100000
10000
1000
100
10
1
SD-1
SD 7
SD 14
SD 20
SD 28
SD 35
SD 43
Study Day
T01-Placebo
T02-cPCV2b Low
T03-cPCV2b High
T04-PCV2b DIV Low
T05-PCV2b DIV High
T06-Comparative

FIG. 6

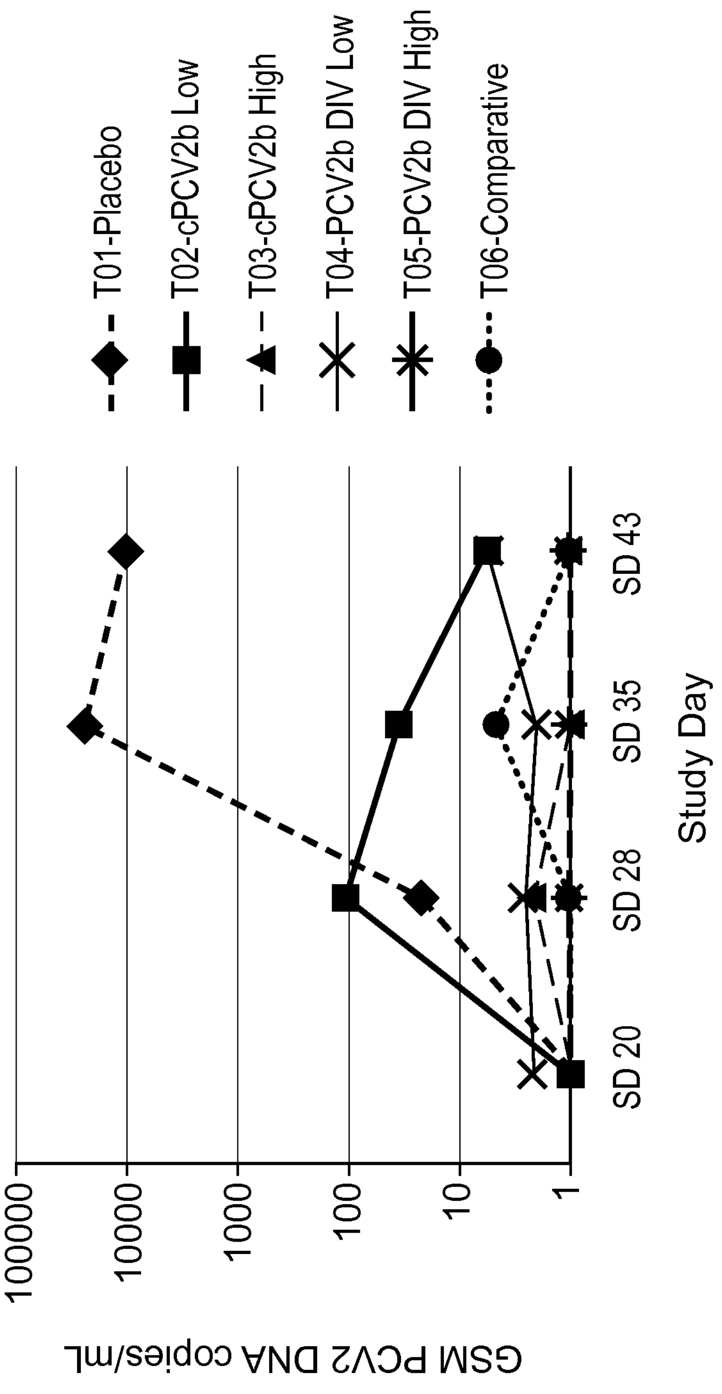
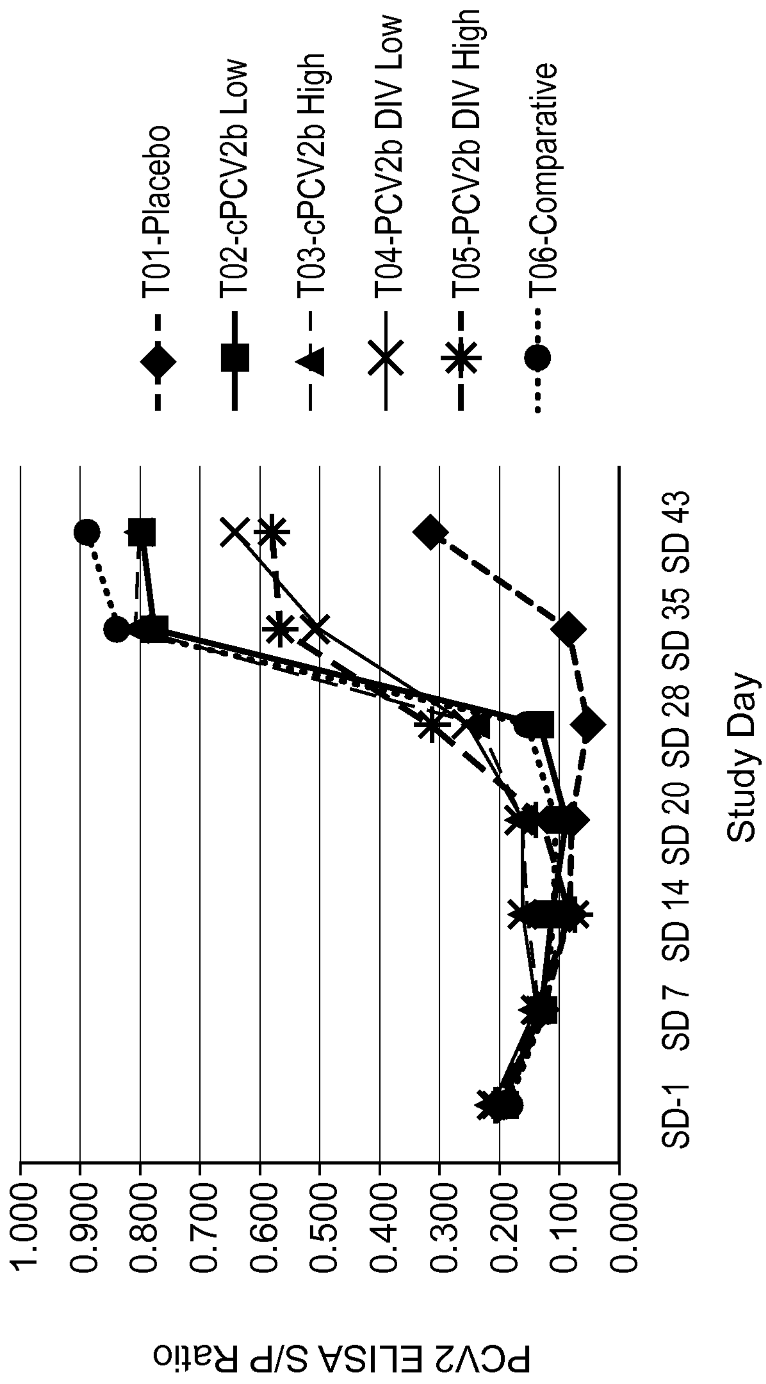

GSM PCV2 DNA copies/mL
100000
10000
1000
100
10
1
SD 20
SD 28
SD 35
SD 43
Study Day
T01-Placebo
T02-cPCV2b Low
T03-cPCV2b High
T04-PCV2b DIV Low
T05-PCV2b DIV High
T06-Comparative

FIG. 7


PCV2 ELISA S/P Ratio
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
SD-1
SD 7
SD 14
SD 20
SD 28
SD 35
SD 43
Study Day
T01-Placebo
T02-cPCV2b Low
T03-cPCV2b High
T04-PCV2b DIV Low
T05-PCV2b DIV High
T06-Comparative

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/057190

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/12 C12N7/00 C12N7/04 A61P31/20
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search, BIOSIS, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CN 102 296 089 A (CHINA INST OF VETERINARY DRUGS CONTROL; BIOTECHNOLOGY RES INST CAAS) 28 December 2011 (2011-12-28) abstract paragraph [0011] - paragraph [0025] paragraph [0039] - paragraph [0040] paragraph [0134] - paragraph [0227]; claims 1-10; sequence 8 & DATABASE Geneseq [Online] 16 February 2012 (2012-02-16), "Porcine circovirus type 2 cap4 protein, SEQ ID 8.", retrieved from EBI accession no. GSP:AZR61798 Database accession no. AZR61798 the whole document ----- -/--	1,4-11, 18-21, 25-27,30 2,3, 12-17, 22-24, 28,29

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 January 2015	03/02/2015

Name and mailing address of the ISA/	Authorized officer
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1

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   [ ] on paper
   [X] in electronic form

   b. (time)

   [X] in the international application as filed
   [ ] together with the international application in electronic form
   [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CN 103 122 352 A (UNIV HUAZHONG AGRICULTURAL) 29 May 2013 (2013-05-29) abstract paragraph [0005] - paragraph [0009] paragraph [0041] - paragraph [0061]; figures 1-7 paragraph [0062] - paragraph [0089] paragraph [0090] - paragraph [0122]; claims 1-4 & DATABASE Geneseq [Online] 19 December 2013 (2013-12-19), "Porcine circovirus 2 ORF2 encoded protein, SEQ ID 2.", retrieved from EBI accession no. GSP:BAY15040 Database accession no. BAY15040 the whole document -----	1,4-11, 18-21, 25-27,30 2,3, 12-17, 22-24, 28,29
Y	WO 2011/116094 A2 (VIRGINIA TECH INTELL PROP [US]; MENG XIANG-JIN [US]; BEACH NATHAN [US]) 22 September 2011 (2011-09-22) abstract page 40, paragraph 0107 - page 41, paragraph 0110; examples 7, 9 -----	1-30
Y	TANJA OPRIESSNIG ET AL: "A PCV2 vaccine based on genotype 2b is more effective than a 2a-based vaccine to protect against PCV2b or combined PCV2a/2b viremia in pigs with concurrent PCV2, PRRSV and PPV infection", VACCINE, vol. 31, no. 3, 1 January 2013 (2013-01-01), pages 487-494, XP055151226, ISSN: 0264-410X, DOI: 10.1016/j.vaccine.2012.11.030 abstract page 488, left-hand column, paragraph 2 - paragraph 4 page 492, left-hand column, paragraph 1 - page 493, left-hand column, paragraph 2; figures 1-3; tables 1-3 ----- -/--	1-30

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BEACH N M ET AL: "Novel chimeric porcine circovirus (PCV) with the capsid gene of the emerging PCV2b subtype cloned in the genomic backbone of the non-pathogenic PCV1 is attenuated in vivo and induces protective and cross-protective immunity against PCV2b and PCV2a subtypes in pigs", VACCINE, ELSEVIER LTD, GB, vol. 29, no. 2, 16 December 2010 (2010-12-16), pages 221-232, XP027539082, ISSN: 0264-410X [retrieved on 2010-10-31] abstract page 222, left-hand column, paragraph 3 page 222, left-hand column, last paragraph - page 223, right-hand column, last paragraph page 229, left-hand column, last paragraph - page 230, left-hand column, paragraph 3; figures 4-7; table 4 -----	1-30
A	TANJA OPRIESSNIG ET AL: "Emergence of a novel mutant PCV2b variant associated with clinical PCVAD in two vaccinated pig farms in the U.S. concurrently infected with PPV2", VETERINARY MICROBIOLOGY, vol. 163, no. 1-2, 1 April 2013 (2013-04-01), pages 177-183, XP055159909, ISSN: 0378-1135, DOI: 10.1016/j.vetmic.2012.12.019 the whole document -----	1-30

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/057190

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
CN 102296089	A	28-12-2011	NONE			
CN 103122352	A	29-05-2013	NONE			
WO 2011116094	A2	22-09-2011	CN	102971425	A	13-03-2013
			EP	2547770	A2	23-01-2013
			US	2011280905	A1	17-11-2011
			US	2014220067	A1	07-08-2014
			WO	2011116094	A2	22-09-2011

SEQUENCE LISTING

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<130> ZP000029A

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<141> 2013-09-25

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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
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Phe Val Thr Lys Ala Asn Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
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Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
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Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Ala Thr Thr Val Arg Thr
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Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
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Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
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Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
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Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
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Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
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Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
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Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr Arg
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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Ala Thr Thr Val Arg Thr
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Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
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Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
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Phe Val Thr Lys Ala Asn Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
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Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile His Asp
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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Ala Thr Thr Val Arg Thr
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Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
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Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
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Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
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Phe Val Thr Lys Ala Asn Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
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Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
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Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
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Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Ala Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
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Phe Val Thr Lys Ala Asn Ala Leu Thr Tyr Asp Pro Tyr Val Asn Tyr
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Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
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Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
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Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
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Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
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Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
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Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
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Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
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Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
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Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
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Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Ile Tyr Asp
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Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Asn Pro
225 230

<210> 16
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 16
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gctgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatag gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa cccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttttagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaccctt aa 702

<210> 17
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 17

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Cys Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Met Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Thr Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro
225   230

<210> 18
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 18
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gctgccgccc ctggctcgtc caccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatgg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca accacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaccctt aa 702

<210> 19
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 19

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Ala Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225 230

<210> 20
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 20
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cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaggctacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata ccccagccct tctcctacca ctcccggtac 480 tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta agtga 705

<210> 21
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 21

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp His Pro Leu Asn Pro Lys
225 230

<210> 22
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 22
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa cccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agaccaccca cttaacccta agtga 705

<210> 23
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 23

Met Thr Tyr Ser Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ser Ala Asn
            180   185   190

Val Asp His Val Gly Leu Gly Thr Ala Phe Glu Asn Ser Lys Tyr Asp
        195   200   205

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 24
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 24
atgacgtatt ccaggaggcg tttccgcaga agaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt gaagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa cccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaacctct gcaaatgtag accacgtagg cctcggcact 600

gcgttcgaaa acagtaaata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agaccccccca cttaacccta aatga 705

<210> 25
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 25

Met Thr Phe Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225 230

<210> 26
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 26
atgacgtttc caaggaggcg ttaccgaaga agaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360 actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctacgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactgt ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta aatga 705

<210> 27
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 27

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Lys Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225 230

<210> 28
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 28
atgacgtatc caaggaggcg ttaccggaaa agaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag gggcctcaaa cccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactgt ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta agtga 705

<210> 29
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 29

Met Thr Tyr Ser Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 30
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 30
atgacgtatt caaggaggcg tttccgcaga agaagacacc gcccccgcag ccatcttggc   60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg   120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc   180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt   240

cccccaggag gggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag   300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc   360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc   420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac   480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga   540

aatcaactgt ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact   600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa   660

ttcagagaat ttaatcttaa agaccccca cttaacccta agtga   705

<210> 31
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 31

Met Thr Tyr Ser Met Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 32
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 32
atgacgtatt caatgaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240 cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta agtga 705

<210> 33
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 33

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 34
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 34
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata ccccagccct tctcctacca ctcccgctat 480

ttcaccccca aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta ag 702

<210> 35

<211> 233
<212> PRT
<213> Porcine circovirus

<400> 35

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro
225   230

<210> 36
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 36
atgacgtatc caaggaggcg ttaccggaga agaagacacc gcccccgcag ccatcttggc   60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg   120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc   180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt   240

cccccaggag gggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag   300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc   360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc   420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac   480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga   540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact   600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtgtaaccat gtatgtacaa   660

ttcagagaat ttaatcttaa agacccccca cttaaccctt aa   702

<210> 37
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 37

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Val Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 38
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 38
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc   60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg   120 aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atggtgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataacgat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta agtga 705

<210> 39
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 39

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Gly Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 40
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 40
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta gcaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgatgggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660 ttcagagaat ttaaccttaa agacccccca cttaacccta agtga 705

<210> 41
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 41

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Lys Pro
225 230

<210> 42
<211> 718
<212> DNA
<213> Porcine circovirus

<400> 42
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaaccct aaatgaataa taaaaacc 718

<210> 43
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 43

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Lys Pro
225   230

<210> 44
<211> 699
<212> DNA
<213> Porcine circovirus

<400> 44

atgacgtatc caaggaggcg tttccgcaga cgaagacacc gccccсgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc caccccсgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag gggctcaaa cccctcact gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaaccc 699

<210> 45
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 45

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Lys Pro
225 230

<210> 46
<211> 699
<212> DNA
<213> Porcine circovirus

<400> 46
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttacccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga 540 aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaaccc 699

<210> 47
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 47

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Gly Pro Pro Leu Lys Pro
225 230

<210> 48
<211> 699
<212> DNA
<213> Porcine circovirus

<400> 48
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag gggctcaaa ccccctcact gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa gggcccccca cttaaaccc 699

<210> 49
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 49

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Gly Pro Pro Leu Lys Pro
225 230

<210> 50
<211> 699

<212> DNA
<213> Porcine circovirus

<400> 50
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc   60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg   120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc   180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt   240

cccccaggag ggggctcaaa cccccctcact gtgccctttg aatactacag aataagaaag   300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc   360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc   420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac   480

tttaccccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga   540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact   600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa   660

ttcagagaat ttaatcttaa gggcccccca cttaaaccc   699

<210> 51
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 51

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Gly Pro Pro Leu Lys Pro
225 230

<210> 52
<211> 699
<212> DNA
<213> Porcine circovirus

<400> 52
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tctttaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag ggggctcaaa ccccctcact gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420 tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa gggcccccca cttaaaccc 699

<210> 53
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 53

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Arg Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Gly Pro Pro Leu Lys Pro
225 230

<210> 54
<211> 699
<212> DNA
<213> Porcine circovirus

<400> 54
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caaaaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag gggctcaaa cccctcact gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgataggaca atcgattact tccaaccaaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa gggcccccca cttaaaccc 699

<210> 55
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 55

Met Thr Tyr Pro Arg Arg Arg Phe Arg Arg Arg Arg His Arg Pro Arg
1   5   10   15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
            20   25   30

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

Leu Ser Arg Thr Ile Gly Tyr Thr Val Lys Lys Thr Thr Val Arg Thr
    50   55   60

Pro Ser Trp Asn Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65   70   75   80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
                85   90   95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
            100   105   110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
        115   120   125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145   150   155   160

Phe Thr Pro Lys Pro Val Leu Asp Gly Thr Ile Asp Tyr Phe Gln Pro
                165   170   175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Thr Gly Asn
            180   185   190

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

Gln Asp Tyr Asn Ile Arg Ile Thr Met Tyr Val Gln Phe Arg Glu Phe
    210   215   220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225   230

<210> 56
<211> 705
<212> DNA
<213> Porcine circovirus

<400> 56
atgacgtatc caaggaggcg tttccgcaga cgaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccatcg gttatactgt caagaaaacc 180

acagtcagaa cgccctcctg gaatgtggac atgatgagat ttaatattaa tgattttctt 240

cccccaggag gggctcaaa cccctcact gtgccctttg aatactacag aataaggaag 300

gttaaggttg aattctggcc ctgctcccca atcacccagg gtgacagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca atgccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccggtac 480

tttaccccga aacctgtcct tgatgggaca atcgattact tccaacccaa taacaaaaga 540

aatcaactct ggctgagact acaaactact ggaaatgtag accatgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtataaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agaccccca cttaacccta agtga 705

<210> 57
<211> 1767
<212> DNA
<213> Porcine circovirus

<400> 57
aaatttctga caaacgttac agggtgctgc tctgcaacgg tcaccagact cccgctctcc 60

aacaaggtac tcacagcagt agacaggtca ctgcgttgtc cttgagatct aggagctcca 120

cactcgataa gtaagttgcc ttctttactg cagtattctt tattctgctg gtctgttcct 180

ttcgctttct cgatgtggca gcgggcacca aaataccact tcactttatt aaaagtttgc 240

ttcttcacaa aattagcgaa cccctgtagg tggggtgttc ggccttcctc attaccctcc 300

tcgccaacaa taaaataatc aaatagggag attgggagct cccgtatttt cttgcgctcg 360

tcttcggaag gattattgag agtgaacacc caccttttat gtggttgggg tccgcttctt 420

ccattcttct tactgggcat gttgctgctg aggtgctgcc gaggtgctgc cgctgccgaa 480

gtgcgctggt aatacttaca gcgcacttct ttcgttttca gctatgacgt atccaaggag 540

gcgtttccgc agacgaagac accgcccccg cagccatctt ggccagatcc tccgccgccg 600 cccctggctc gtccaccccc gccaccgtta ccgctggaga aggaaaaatg gcatcttcaa 660

cacccgcctc tcccgcacca tcggttatac tgtcaagaaa accacagtca gaacgccctc 720

ctggaatgtg gacatgatga gatttaatat taatgatttt cttcccccag gagggggctc 780

aaaccccctc actgtgccct ttgaatacta cagaataagg aaggttaagg ttgaattctg 840

gccctgctcc ccaatcaccc agggtgacag gggagtgggc tccactgctg ttattctaga 900

tgataacttt gtaacaaagg ccaatgccct aacctatgac ccctatgtaa actactcctc 960

ccgccatacc ataacccagc ccttctccta ccactcccgg tactttaccc cgaaacctgt 1020

ccttgatggg acaatcgatt acttccaacc caataacaaa agaaatcaac tctggctgag 1080

actacaaact actggaaatg tagaccatgt aggcctcggc actgcgttcg aaaacagtat 1140

atacgaccag gactacaata tccgtataac catgtatgta caattcagag aatttaatct 1200

taaagacccc ccacttaacc ctaagtgaat aataaaaacc attacgaagt gataaaaaag 1260

actcagtaat ttatttcata tggaaattca gggcatgggg gggaaagggt gacgaactgg 1320

ccccttcct ccgtggattg ttctgtagca ttcttccaaa ataccaagga agtaatcctc 1380

cgatagagag cttctacagc taggacagca gttgaggagt accattccaa cggggtctga 1440

ttgctggtaa tcagaatact gcgggccaaa aaaggtacag ttccaccttt agtctctaca 1500

gtcaatggat atcgatcaca cagtctcagt agatcatccc acggcagcca accataaaag 1560

tcatcaataa caaccacttc ttcaccatgg taaccatccc accacttgtt tcgaggtggt 1620

ttccagtatg tggtttccgg gtctgcaaaa ttagcagccc atttgctttt accacaccca 1680

ggtggcccca caatgacgtg tacattggtc ttccaatcac gcttctgcat tttcccgctc 1740

actttcaaaa gttcagccag cccgcgg 1767

<210> 58
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 58

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Thr Arg

35 40 45

Leu Ser Arg Thr Phe Gly Tyr Thr Val Lys Ala Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asp Asp Val Val
65 70 75 80

Pro Pro Gly Gly Gly Thr Asn Lys Ile Ser Ile Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Met Arg Leu Gln Thr Ser Arg Asn
180 185 190

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

Gln Asp Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Lys Pro
225 230

<210> 59
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 59
atgacgtatc caaggaggcg ttaccgcaga agaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgctaccg ttggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccttcg gatatactgt caaggctacc 180

acagtcagaa cgccctcctg ggcggtggac atgatgagat ttaatattga cgactttgtt 240

cccccgggag gggggaccaa caaaatctct ataccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctccccc atcacccagg gtgatagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca cagccctaac ctatgaccca 420

tatgtaaact actcctcccg ccatacaatc ccccaaccct tctcctacca ctcccgttac 480

ttcacaccca aacctgttct tgactccacc attgattact tccaaccaaa taacaaaagg 540

aatcagcttt ggatgaggct acaaacctct agaaatgtgg accacgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggac tacaatatcc gtgtaaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaaccct aa 702

<210> 60
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 60

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Leu His Pro
20 25 30

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

Leu Ser Arg Thr Phe Gly Tyr Thr Val Lys Arg Thr Thr Val Thr Thr
50 55 60

Pro Ser Trp Ala Val Asp Met Met Arg Phe Lys Ile Asp Asp Phe Val
65 70 75 80

Pro Pro Gly Gly Gly Thr Asn Lys Ile Ser Ile Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Pro Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ser Gly Asn
180 185 190

Val Asp His Val Gly Leu Gly Ile Ala Phe Glu Asn Ser Lys Tyr Asp
195 200 205

Gln Asp Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Lys Pro
225 230

<210> 61
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 61
atgacgtatc caaggaggcg ttaccgcaga agaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcctc cacccccgcc accgctaccg ttggagaagg 120

aaaaatggca tcttcaacac ccgcctctcc cgcaccttcg gatatactgt caagcgtacc 180

acagtcacaa cgccctcctg ggcggtggac atgatgagat ttaaaattga cgactttgtt 240

cccccgggag gggggaccaa caaaatctct ataccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctccccc atcacccagg gtgatagggg agtgggctcc 360

actgctgtta ttctagatga taactttgta acaaaggcca cagccctaac ctatgaccca 420

tatgtaaact actcctcccg ccatacaatc ccccaaccct tctcctacca ctcccgttac 480

ttcacaccca aacctgttct tgactccact attgattact tccaaccaaa taacaaaagg 540

aatcagcttt ggctgaggct acaaacctct ggaaatgtgg accacgtagg cctcggcatt 600 gcgttcgaaa acagtaaata cgaccaggac tacaatatcc gtgtaaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaaaccct aa 702

<210> 62
<211> 233
<212> PRT
<213> Porcine circovirus

<400> 62

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly Gln Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro
20 25 30

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

Leu Ser Arg Thr Phe Gly Tyr Thr Ile Lys Arg Thr Thr Val Arg Thr
50 55 60

Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asn Asp Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Arg Ser Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Trp Pro Cys Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Ser Ala Val Ile Leu Asp Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Leu Arg Leu Gln Thr Ala Gly Asn

180 185 190

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

Gln Glu Tyr Asn Ile Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Asn Pro
225 230

<210> 63
<211> 702
<212> DNA
<213> Porcine circovirus

<400> 63
atgacgtatc caaggaggcg ttaccggaga agaagacacc gcccccgcag ccatcttggc 60

cagatcctcc gccgccgccc ctggctcgtc cacccccgcc accgttaccg ctggagaagg 120

aaaaatggca tcttcaacac ccgcctatcc cgcaccttcg gatatactat caagcgaacc 180

acagtcagaa cgccctcctg ggcggtggac atgatgagat tcaatattaa tgactttctt 240

cccccaggag gggctcaaa cccccgctct gtgccctttg aatactacag aataagaaag 300

gttaaggttg aattctggcc ctgctccccg atcacccagg gtgacagggg agtgggctcc 360

agtgctgtta ttctagatga taactttgta acaaaggcca cagccctcac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccagccct tctcctacca ctcccgctac 480

tttaccccca aacctgtcct agattccact attgattact tccaaccaaa caacaaaaga 540

aaccagctgt ggctgagact acaaactgct ggaaatgtag accacgtagg cctcggcact 600

gcgttcgaaa acagtatata cgaccaggaa tacaatatcc gtgtaaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agaccccccca cttaaccctt aa 702

<210> 64
<211> 234
<212> PRT
<213> Porcine circovirus

<400> 64

Met Thr Tyr Pro Arg Arg Arg Tyr Arg Arg Arg Arg His Arg Pro Arg
1 5 10 15

Ser His Leu Gly His Ile Leu Arg Arg Arg Pro Trp Leu Val His Pro

20 25 30

Arg His Arg Tyr Arg Trp Arg Arg Lys Asn Gly Ile Phe Asn Ala Arg
35 40 45

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

Pro Ser Trp Ala Val Asp Met Met Arg Phe Asn Ile Asn Gln Phe Leu
65 70 75 80

Pro Pro Gly Gly Gly Ser Asn Pro Leu Thr Val Pro Phe Glu Tyr Tyr
85 90 95

Arg Ile Arg Lys Val Lys Val Glu Phe Phe Ala Arg Ser Pro Ile Thr
100 105 110

Gln Gly Asp Arg Gly Val Gly Ser Thr Ala Val Ile Leu Asn Asp Asn
115 120 125

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

Ser Ser Arg His Thr Ile Thr Gln Pro Phe Ser Tyr His Ser Arg Tyr
145 150 155 160

Phe Thr Pro Lys Pro Val Leu Asp Ser Thr Ile Asp Tyr Phe Gln Pro
165 170 175

Asn Asn Lys Arg Asn Gln Leu Trp Met Arg Leu Gln Thr Thr Gly Asn
180 185 190

Val Asp His Val Gly Leu Gly His Ala Phe Gln Asn Ser Thr Asn Ala
195 200 205

Gln Ala Tyr Asn Val Arg Val Thr Met Tyr Val Gln Phe Arg Glu Phe
210 215 220

Asn Leu Lys Asp Pro Pro Leu Asn Pro Lys
225 230

<210> 65
<211> 705
<212> DNA

<213> Porcine circovirus

<400> 65
atgacgtatc caaggaggcg ttaccggaga agaagacacc gcccccgcag ccatcttggc 60

catatcctcc gccgccgccc ctggctcgtc cacccccgcc accgctaccg ttggagaagg 120

aaaaatggaa tcttcaatgc ccgcctctcc cgctcctttg tttataccgt taatgcctca 180

caggtctcac caccctcttg ggcggtggac atgatgagat ttaatattaa ccaatttctt 240

cccccaggag gggctcaaa cccctcact gtgccctttg aatactacag aataagaaag 300

gttaaagtgg aattctttgc aagatccccc atcacccaag gtgacagggg agtgggctcc 360

actgctgtta ttctaaatga taactttgta acaaaggcca cagccctaac ctatgacccc 420

tatgtaaact actcctcccg ccataccata acccaaccct tctcctacca ctcccgctac 480

tttacccca aacctgtcct tgattccact attgattact tccaaccaaa taacaaaaga 540

aatcagctgt ggatgagact acaaactact ggaaatgtag accatgtagg cctcggacac 600

gcctttcaaa acagtacaaa tgcccaggcc tacaatgtcc gtgtaaccat gtatgtacaa 660

ttcagagaat ttaatcttaa agacccccca cttaacccta agtga 705

<210> 66
<211> 1767
<212> DNA
<213> Porcine circovirus

<400> 66
gtcttttta tcacttcgta atggttttta ttattcactt agggttaagt gggggtctt 60

taagattaaa ttctctgaat tgtacataca tggttatacg gatattgtag tcctggtcgt 120

atatactgtt ttcgaacgca gtgccgaggc ctacatggtc tacatttcca gtagtttgta 180

gtctcagcca gagttgattt cttttgttat tgggttggaa gtaatcgatt gtcctatcaa 240

ggacaggttt cggggtaaag taccgggagt ggtaggagaa gggctgggtt atggtatggc 300

gggaggagta gtttacatag gggtcatagg ttagggcatt ggcctttgtt acaaagttat 360

catctagaat aacagcagtg gagcccactc ccctgtcacc ctgggtgatt ggggagcagg 420

gccagaattc aaccttaacc ttccttattc tgtagtattc aaagggcaca gtgagggggt 480

ttgagccccc tcctgggga agaaaatcat taatattaaa tctcatcatg tccacattcc 540

aggagggcgt tctgactgtg gttttcttga cagtataacc gatggtgcgg gagaggcggg 600

tgttgaagat gccatttttc cttctccagc ggtaacggtg gcggggtgg acgagccagg 660

ggcggcggcg gaggatctgg ccaagatggc tgcgggggcg gtgtcttcgt ctgcggaaac 720 gcctccttgg atacgtcatc gctgaaaacg aaagaagtgc gctgtaagta ttaccagcgc 780

acttcggcag cggcagcacc tcggcagcac ctcagcagca acatgcccag caagaagagt 840

ggaagaagcg gaccccaacc acataaaagg tgggtgttca cgctgaataa tccttccgaa 900

gacgagcgca agaaaatacg ggagctccca atctccctat ttgattattt tattgttggc 960

gaggaaggta atgaggaggg ccgaacaccg cacctacagg ggttcgctaa ttttgtgaag 1020

aagcaaactt ttaataaagt gaagtggtat tttggtgccc gctgccacat cgagaaagcg 1080

aaaggaacag atcagcagaa taaagaatat tgcagtaaag aaggcaactt actgatagaa 1140

tgtggagctc ctagatctca aggacaacgg agtgacctct ctactgctgt gagtaccttg 1200

ttggagagcg ggagtctggt gaccgttgca gagcagcacc ctgtaacgtt tgtcagaaat 1260

ttccgcgggc tggctgaact tttgaaagtg agcgggaaaa tgcagaagcg tgattggaag 1320

acgaatgtac acgtcattgt ggggccacct gggtgtggca aaagcaaatg ggctgctaat 1380

tttgcagacc cggaaaccac atactggaaa ccacctagaa acaagtggtg ggatggttac 1440

catggtgaag aagtggttgt tattgatgac ttttatggct ggctgccgtg ggatgatcta 1500

ctgagactct gtgatcgata tcctttgact gttgagacta aaggtggaac tgtacctttt 1560

ttggcccgca gtattctgat taccagcaat cagaccccgt tggaatggta ctcctcaact 1620

gctgtcccag ctgtagaagc tctctatcgg aggattactt ccttggtatt ttggaagaat 1680

gctacagaac aatccacgga ggaagggggc cagttcgtca ccctttcccc cccatgccct 1740

gaatttccat atgaaataaa ttactga 1767

摘要

本发明提供了一种用于保护猪对抗PCV2的疫苗组合物，所述疫苗组合物包括高毒力猪2b型圆环病毒(PCV2b)趋异株，所述组合物包括PCV2b趋异株ORF2多肽，其中根据本文的SEQ ID NO:1的编号，所述ORF2多肽包含位置89处的亮氨酸(L)、位置90处的苏氨酸(T)、以及位置134处的天冬酰胺(N)。