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(54) **Infectious cDNA clone of north american porcine reproductive and respiratory syndrome (PRRS) virus and uses thereof**

Infektiöser cDNA Klon des das Reproduktions- und Atemwegs-Syndrom verursachenden Nord-Amerikanischen Schweinevirus und dessen Verwendung

Clone de cDNA du virus du syndrome dysgénésique respiratoire porcin (SDRP) d'Amérique du Nord et méthodes d'utilisation

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- **DATABASE EMBL [Online] U87392, PRSS virus strain VR-2332, compl. genome, 8 January 1998 (1998-01-08) XP002189646**
- **DATABASE EMBL [Online] AF046869, PRRS virus 16244B, complete genome, 21 October 1998 (1998-10-21) XP002189647**
- **MEULENBERG ET AL: "Infectious transcripts from cloned genome-length cDNA of Porcine Reproductive and Respiratory Syndrome Virus" JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 72, no. 1, January 1998 (1998-01), pages 380-387, XP002112766 ISSN: 0022-538X**

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- YANG S X ET AL: "Comparative sequence analysis of open reading frames 2 to 7 of the modified live vaccine virus and other North American isolates of the porcine reproductive and respiratory syndrome virus" ARCHIVES OF VIROLOGY, NEW YORK, NY, US, vol. 143, no. 3, 1998, pages 601-612, XP000926565 ISSN: 0304-8608
- KAPUR V ET AL: "GENETIC VARIATION IN PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS ISOLATES IN THE MIDWESTERN UNITED STATES" JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 77, 1996, pages 1271-1276, XP002919855 ISSN: 0022-1317
- SNIJDER E J ET AL: "THE MOLECULAR BIOLOGY OF ARTERIVIRUSES" JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 29, 1998, pages 961-979, XP000791110 ISSN: 0022-1317

- DATABASE EMBL [Online] AF066183, RespPRRS MLV, complete genome, 27 April 1999 (1999-04-27) XP002189648
- DATABASE EMBL [Online] AF66384, PRRS virus, partial sequence, 27 April 1999 (1999-04-27) XP002189649

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description**Field of the Invention**

- 5 **[0001]** The present invention is in the field of animal health and is directed to infectious cDNA clones of positive polarity RNA viruses and the construction of vaccines, in particular, swine vaccines, using such cDNA clones.

Background of the Invention

- 10 **[0002]** Porcine reproductive and respiratory syndrome (PRRS) is a new disease of swine, first described in 1987 in North America and in 1990 in Europe. The disease has since spread to Asia and affects most of the major swine producing countries of the world. Primary symptoms are reproductive problems in sows and gilts, including late term abortions, stillbirths and mummies, and litters of small weak pigs which are born viremic and often fail to survive. In addition, the syndrome manifests itself as a respiratory disease in young pigs which spreads horizontally and causes fever, lethargy, 15 labored breathing, loss of appetite, slow growth, and occasionally death, often in association with other respiratory pathogens. The disease furthermore can be transmitted to sows and gilts via the semen of infected boars, either naturally or by artificial insemination. For these, and other reasons, PRRS has proven to be a difficult disease to control and therefore one of the most economically damaging diseases to the swine industry.

- 20 **[0003]** The causative agent of PRRS is the PRRS virus, which exists as two genetically and serologically distinct types (Murtaugh, M.P. *et al.*, 1995, Arch-Virol. 140, 1451-1460; Suarez, P. *et al.*, 1996, Virus Research 42:159-165). The two types are believed to have first entered swine populations independently, one in North America and the other in Europe, in the 1980's, from unknown biological reservoirs, possibly of rodent or avian origin. The European type, represented by the prototype "Lelystad Virus", was isolated and sequenced in the Netherlands in 1991 (Terpstra, C. *et al.*, 1991, Vet. Quart. 13:131-136; Wensvoort, G. *et al.*, 1991, Vet. Quart. 13:121-130; Wensvoort, G. *et al.*, WO 92/213751992 25 (PCT/NL92/00096), 1992; Meulenbergh, J.J.M. *et al.*, 1993, Virol. 192:62-72).

- [0004]** Both the North American PRRS virus and the European PRRS virus are classified within the family *Arteriviridae*, which also includes equine arteritis virus, lactate dehydrogenase-elevating virus, and simian haemorrhagic fever virus. The arteriviruses are in turn placed within the order *Nidovirales*, which also includes the coronaviruses and toroviruses. The nidoviruses are enveloped viruses having genomes consisting of a single strand of positive polarity RNA. The genomic RNA of a positive-stranded RNA virus fulfills the dual role in both storage and expression of genetic information. No DNA is involved in replication or transcription in nidoviruses. The reproduction of nidoviral genomic RNA is thus a combined process of genome replication and mRNA transcription. Moreover, some proteins are translated directly from the genomic RNA of nidoviruses. The molecular biology of the family *Arteriviridae* has recently been reviewed by Snijder and Meuldenbergh (Snijder, E.J. and Meulenbergh, J.J.M., 1998, Journal of General Virology 79:961-979). 30

- 35 **[0005]** Currently available commercial vaccines against PRRS are either conventional modified live virus (cell culture, attenuated) or conventional killed (inactivated cell culture preparations of virulent virus). Several of these vaccines have been criticized based on safety and/or efficacy concerns. The development of a second generation of PRRS vaccines, based upon specific additions, deletions, and other modifications to the PRRS genome, is therefore highly desirable. However, since the PRRS viruses do not include any DNA intermediates during their replication, such vaccines have 40 thus far awaited the construction of full-length cDNA clones of PRRS viruses for manipulation by molecular biology techniques at the DNA level. Very recently, a full-length infectious cDNA clone of the European PRRS virus has been reported (Meulenbergh, J.J.M. *et al.*, 1998, *supra*; Meulenbergh, J.J.M. *et al.*, 1988, J. Virol. 72, 380-387).

[0006] Database EMBL (Online) U87392 discloses the complete genome of PRSS virus strain VR-2332.

[0007] Database EMBL (Online) AF046869 discloses the complete genome of PRRS virus 16244B.

- 45 **[0008]** WO 96/04010 discloses a partial sequence of PRRS virus VR-2332.

Summary of the Invention

- 50 **[0009]** The subject invention provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine.

- 55 **[0010]** The present invention further provides a transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, which transfected host cell is

capable of expressing the encoded North American PRRS virus.

[0011] The present invention further provides a plasmid capable of directly transfecting a suitable host cell and expressing a North American PRRS virus from the suitable host cell so transfected, which plasmid comprises a) a DNA sequence encoding an infectious RNA molecule encoding the North American PRRS virus, and wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, and b) a promoter capable of transcribing said infectious RNA molecule in said suitable host cell.

[0012] The present invention further provides a method for generating a North American PRRS virus, which method comprises transfecting a suitable host cell with a plasmid of the invention encoding the North American PRRS virus and obtaining the North American PRRS virus generated by the transfected host cell.

[0013] The present invention further provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that when it infects a porcine animal it is unable to produce PRRS in the animal, wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, except for that it contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS.

[0014] The present invention further provides a transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that when it infects a porcine animal it is unable to produce PRRS in the animal, wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, except for that it contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS, which transfected host cell is capable of expressing the encoded genetically modified North American PRRS virus.

[0015] The present invention further provides a vaccine for protecting a porcine animal from infection by a PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule of the invention, or said infectious RNA molecule, or said polynucleotide molecule in the form of a plasmid, or a viral vector comprising a nucleotide sequence of the invention encoding a North American PRRS virus that is able to elicit an effective immunoprotective response against infection by a PRRS virus, in an amount effective to produce immunoprotection against infection by a PRRS virus; and a carrier acceptable for veterinary use.

[0016] The present invention further provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more heterologous antigenic epitopes, wherein the DNA sequence encoding the RNA molecule encoding the North American PRRS virus is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, except for that it comprises one or more further nucleotide sequences that each encode a heterologous antigenic epitope, and wherein each heterologous antigenic epitope is capable of inducing an effective immunoprotective response against a particular pathogen in a mammal or a bird.

[0017] The present invention further provides a transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more heterologous antigenic epitopes, wherein the DNA sequence encoding the RNA molecule encoding the North American PRRS virus is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, except for that it comprises one or more further nucleotide sequences that each encode a heterologous antigenic epitope, and wherein each heterologous antigenic epitope is capable of inducing an effective immunoprotective response against a particular pathogen in a mammal or a bird.

[0018] The present invention further provides a vaccine for protecting a mammal or a bird from infection by a pathogen, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule of the invention, or said infectious RNA molecule, or a plasmid of the invention, in an amount effective to produce immunoprotection against infection by the pathogen from which the heterologous antigenic epitope or epitopes thereof or encoded thereby are derived; and a carrier acceptable for pharmaceutical or veterinary use.

[0019] The present invention further provides a vaccine for protecting a porcine animal from infection by a PRRS virus and from infection by a swine pathogen other than a North American PRRS virus, which vaccine comprises a genetically

modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule of the invention, or said infectious RNA molecule, or a plasmid according to the invention, in an amount effective to produce immunoprotection against infection by a PRRS virus and against infection by the pathogen from which the heterologous antigenic epitope or epitopes thereof or encoded thereby are derived; and a carrier acceptable for veterinary use.

[0020] The present invention further provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more detectable heterologous antigenic epitopes, wherein the DNA sequence encoding the infectious RNA molecule is the same as the sequence SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, except for that it comprises one or more further nucleotide sequences that each encode a detectable heterologous antigenic epitope.

[0021] The present invention further provides a vaccine for protecting a porcine animal from infection by a PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule of the present invention, comprising one or more detectable heterologous antigenic epitopes, treated or further genetically modified so that it is unable to produce PRRS in a porcine animal yet able to elicit an effective immunoprotective response against a PRRS virus in the porcine animal; or said infectious RNA molecule, wherein the genetically modified North American PRRS virus encoded thereby comprising one or more detectable heterologous antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or an isolated polynucleotide of the present invention, in the form of a plasmid, wherein the genetically modified North American PRRS virus encoded thereby comprising one or more detectable heterologous antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or a viral vector comprising a nucleotide sequence encoding an infectious RNA molecule encoding such a genetically modified North American PRRS virus, wherein the genetically modified North American PRRS virus encoded thereby comprising one or more detectable heterologous antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; in an amount effective to produce immunoprotection against infection by a PRRS virus; and a carrier acceptable for veterinary use.

[0022] The present invention further provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified so that it lacks a detectable antigenic epitope, wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, except for that it lacks one or more DNA sequences encoding a detectable antigenic epitope.

[0023] The present invention further provides a vaccine for protecting a porcine animal from infection by a PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by an polynucleotide molecule of the invention, lacking one or more detectable antigenic epitopes, treated or further genetically modified so that it is unable to produce PRRS in a porcine animal yet able to elicit an effective immunoprotective response against a PRRS virus in the porcine animal; or said infectious RNA molecule, wherein the genetically modified North American PRRS virus encoded thereby lacking one or more detectable antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or an isolated polynucleotide molecule, in the form of a plasmid, wherein the genetically modified North American PRRS virus encoded thereby lacking one or more detectable antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or a viral vector comprising a nucleotide sequence encoding an infectious RNA molecule encoding such a genetically modified North American PRRS virus, wherein the genetically modified North American PRRS virus encoded thereby lacking one or more detectable antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus, in an amount effective to produce immunoprotection against infection by a PRRS virus; and a carrier acceptable for veterinary use.

[0024] The present invention further provides an isolated polynucleotide molecule comprising one or more nucleotide sequences that each encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA molecule corresponding to SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine.

[0025] The present invention further provides a transfected host cell comprising one or more nucleotide sequences

that each encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA molecule corresponding to SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, which transformed cell is capable of expressing said peptide or peptides.

[0026] The present invention further provides a method for preparing a genetically modified North American PRRS virus that is capable of eliciting an immunoprotective response in a mammal or a bird vaccinated therewith, which method comprises obtaining an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a wild-type North American PRRS virus wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15, 416 of SEQ ID NO: 1, except that the nucleotide corresponding to nucleotide 12, 622 of SEQ ID NO: 1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1, 559 of SEQ ID NO: 1 is a thymine instead of a cytosine, genetically mutating the DNA sequence encoding the infectious RNA molecule encoding the wild-type North American PRRS virus so as to obtain an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a genetically modified North American PRRS virus, which virus remains able to elicit an effective immunoprotective response against infection by the wild-type North American PRRS virus in a mammal or a bird, and expressing the genetically modified North American PRRS virus from the isolated polynucleotide molecule so obtained.

[0027] The present invention further provides a vaccine for protecting a mammal or a bird from infection by a North American PRRS virus, which comprises a genetically modified North American PRRS virus of the invention in an amount effective to elicit an effective immunoprotective response against the wild-type North American PRRS virus in a mammal or a bird vaccinated therewith, and a carrier acceptable for pharmaceutical or veterinary use.

Brief Description of the Figures

[0028]

FIGURE 1: Cloning strategy for construction of full-length infectious cDNA clone of North American PRRS virus, pT7P129A. Arrowheads represent T7 promoter sequences.

FIGURE 2: Serum viremia following infection with P129A or recombinant PRRS virus rP129A-1. Determined by plaque assay on MARC-145 cells. The lower limit of detection is 5 pfu/ml (or 0.7 on the log scale)..

FIGURE 3: Anti-PRRS virus serum antibody following infection with P129A or recombinant PRRS virus rP129A-1. Determined by HerdChek PRRS ELISA assay (IDEXX (Westbrook, Maine, USA)).

Detailed Description of the Invention

[0029] Production and manipulation of the isolated polynucleotide molecules described herein are within the skill in the art and can be carried out according to recombinant techniques described, among other places, in Maniatis, *et al.*, 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel, *et al.*, 1989, Current Protocols In Molecular Biology, Greene Publishing Associates & Wiley Interscience, NY; Sambrook, *et al.*, 1989, Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Innis *et al.* (eds), 1995, PCR Strategies, Academic Press, Inc., San Diego; and Erlich (ed), 1992, PCR Technology, Oxford University Press, New York.

[0030] A. *Isolated Polynucleotide Molecules and RNA Molecules Encoding a North American PRRS Virus, and Isolated Polynucleotide Molecules and RNA Molecules Encoding Genetically Modified North American PRRS Viruses:*

[0031] The subject invention provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule that encodes a North American PRRS virus, wherein said DNA sequence is SEQ ID NO:1 In a preferred embodiment, the present invention provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule that encodes a North American PRRS virus, wherein said DNA sequence is the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine. Said DNA sequence encodes an infectious RNA molecule that is the RNA genome of the North American PRRS isolate P129.

[0032] It is understood that terms herein referring to nucleic acid molecules such as "isolated polynucleotide molecule", "nucleotide sequence", "open reading frame (ORF)", and the like, unless otherwise specified, include both DNA and RNA molecules and include both single-stranded and double-stranded molecules. Also, when reference to a particular sequence from the "Sequence Listing" section of the subject application is made, it is intended, unless otherwise specified, to refer to both the DNA of the "Sequence Listing", as well as RNA corresponding to the DNA sequence, and includes

sequences complementary to the DNA and RNA sequences. In such contexts in this application, "corresponding to" refers to sequences of DNA and RNA that are identical to one another but for the fact that the RNA sequence contains uracil in place of thymine and the backbone of the RNA molecule contains ribose instead of deoxyribose.

[0033] For example, SEQ ID NO:1 is a DNA sequence corresponding to the RNA genome of a North American PRRS virus. Thus, a DNA sequence complementary to the DNA sequence set forth in SEQ ID NO:1 is a template for, *i.e.* is complementary to or "encodes", the RNA genome of the North American PRRS virus (*i.e.*, RNA that encodes the North American PRRS virus). Nonetheless, a reference herein to SEQ ID NO:1 includes both the RNA sequence corresponding to SEQ ID NO:1 and a DNA sequence complementary to SEQ ID NO:1.

[0034] An "infectious RNA molecule", for purposes of the present invention, is an RNA molecule that encodes the necessary elements for viral replication, transcription, and translation into a functional virion in a suitable host cell, provided, if necessary, with a peptide or peptides that compensate for any genetic modifications, *e.g.* sequence deletions, in the RNA molecule.

[0035] An "isolated infectious RNA molecule" refers to a composition of matter comprising the aforementioned infectious RNA molecule purified to any detectable degree from its naturally occurring state, if such RNA molecule does indeed occur in nature. Likewise, an "isolated polynucleotide molecule" refers to a composition of matter comprising a polynucleotide molecule of the present invention purified to any detectable degree from its naturally occurring state, if any.

[0036] It is furthermore to be understood that the isolated polynucleotide molecules and the isolated RNA molecules of the present invention include both synthetic molecules and molecules obtained through recombinant techniques, such as by *in vitro* cloning and transcription.

[0037] As used herein, the term "PRRS" encompasses disease symptoms in swine caused by a PRRS virus infection. Examples of such symptoms include, but are not limited to, abortion in pregnant females, and slow growth, respiratory difficulties, loss of appetite, and mortality in young pigs. As used herein, a PRRS virus that is "unable to produce PRRS" refers to a virus that can infect a pig, but which does not produce any disease symptoms normally associated with a PRRS infection in the pig, or produces such symptoms, but to a lesser degree, or produces a fewer number of such symptoms, or both.

[0038] The terms "porcine" and "swine" are used interchangeably herein and refer to any animal that is a member of the family Suidae such as, for example, a pig. "Mammals" include any warm-blooded vertebrates of the Mammalia class, including humans.

[0039] The term "PRRS virus", as used herein, unless otherwise indicated, means any strain of either the North American or European PRRS viruses.

[0040] The term "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-1 b (Kwang, J. *et al.*, 1994, J. Vet. Diagn. Invest. 6:293-296); the Quebec IAF-exp91 strain of PRRS (Mardassi, H, *et al.*, 1995, Arch. Virol. 140:1405-1418); and North American PRRS virus isolate VR 2385 (Merig, X.-J *et al.*, 1994, J. Gen. Virol. 75:1795-1801). Genetic characteristics refers to genomic nucleotide sequence similarity and aminoacid sequence similarity shared by North American PRRS virus strains. For purposes of the present invention, a North American PRRS virus is a virus that is encoded by an RNA sequence the same as SEQ ID NO:1.

[0041] The term "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".

[0042] The term "open reading frame", or "ORF", as used herein, means the minimal nucleotide sequence required to encode a particular PRRS virus protein without an intervening stop codon.

[0043] Terms such as "suitable host cell" and "appropriate host cell", unless otherwise indicated, refer to cells into which RNA molecules (or isolated polynucleotide molecules or viral vectors comprising DNA sequences encoding such RNA molecules) of the present invention can be transformed or transfected. "Suitable host cells" for transfection with such RNA molecules, isolated polynucleotide molecules, or viral vectors, include mammalian, particularly porcine, and avian cells, and are described in further detail below.

[0044] A "functional virion" is a virus particle that is able to enter a cell capable of hosting a PRRS virus, and express genes of its particular RNA genome (either an unmodified genome or a genetically modified genome as described herein) within the cell. Cells capable of hosting a PRRS virus include porcine alveolar macrophage cells and MARC 145 monkey kidney cells. Other mammalian or avian cells, especially other porcine cells, may also serve as suitable host cells for PRRS virions.

[0045] The isolated polynucleotide molecules of the present invention encode North American PRRS viruses that can be used to prepare live, killed, or attenuated vaccines using art-recognized methods for protecting swine from infection by a PRRS virus, as described in further detail below. These isolated polynucleotide molecules are also useful as vectors for delivering heterologous genes into mammals, including swine, or birds, as is also described in detail below. Furthermore, these isolated polynucleotide molecules are useful because they can be mutated using molecular biology tech-

niques to encode genetically-modified North American PRRS viruses useful, *inter alia*, as vaccines for protecting swine from PRRS infection. Such genetically-modified North American PRRS viruses, as well as vaccines comprising them, are also described in further detail below.

[0046] Accordingly, the subject invention further provides a method for making a genetically modified North American PRRS virus, which method comprises mutating the DNA sequence encoding an infectious RNA molecule which encodes the North American PRRS virus as described above, and expressing the genetically modified North American PRRS virus using a suitable expression system. A North American PRRS virus, either wild-type or genetically modified, can be expressed from an isolated polynucleotide molecule using suitable expression systems generally known in the art, examples of which are described in this application. For example, the isolated polynucleotide molecule can be in the form of a plasmid capable of expressing the encoded virus in a suitable host cell *in vitro*, as is described in further detail below.

[0047] The term "genetically modified", as used herein and unless otherwise indicated, means genetically mutated, *i.e.* having one or more nucleotides replaced, deleted and/or added. Polynucleotide molecules can be genetically mutated using recombinant techniques known to those of ordinary skill in the art, including by site-directed mutagenesis, or by random mutagenesis such as by exposure to chemical mutagens or to radiation, as known in the art. In one embodiment, genetic modification of the North American PRRS virus of the present invention renders the virus unable to replicate effectively, or reduces its ability to replicate effectively, in a bird or mammal in which the wild-type virus otherwise can effectively replicate. In another embodiment, the genetically modified North American PRRS virus of the present invention remains able to replicate effectively in birds or mammals infected therewith. "Effective replication" means the ability to multiply and produce progeny viruses (virions) in an infected animal, *i.e.* the ability to "productively infect" an animal.

[0048] The subject invention further provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule which encodes a genetically modified North American PRRS virus that is unable to produce PRRS in a porcine animal, wherein the DNA sequence encoding the infectious RNA molecule encoding said North American PRRS virus is SEQ ID NO:1 except that it contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS. "Genetically disabled" means that the PRRS virus is unable to produce PRRS in a swine animal infected therewith.

[0049] In one embodiment, the genetically modified North American PRRS virus disabled in its ability to cause PRRS is able to elicit an effective immunoprotective response against infection by a PRRS virus in a swine animal. Accordingly, the subject invention also provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule which encodes a North American PRRS virus that is genetically modified such that when it infects a porcine animal it: a) is unable to produce PRRS in the animal, and b) is able to elicit an effective immunoprotective response against infection by a PRRS virus in the animal, wherein the DNA sequence encoding said North American PRRS virus is SEQ ID NO:1 except that it contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS.

[0050] The term "immune response" for purposes of this invention means the production of antibodies and/or cells (such as T lymphocytes) that are directed against, or assist in the decomposition or inhibition of, a particular antigenic epitope or particular antigenic epitopes. The phrases "an effective immunoprotective response", "immunoprotection", and like terms, for purposes of the present invention, mean an immune response that is directed against one or more antigenic epitopes of a pathogen so as to protect against infection by the pathogen in a vaccinated animal. For purposes of the present invention, protection against infection by a pathogen includes not only the absolute prevention of infection, but also any detectable reduction in the degree or rate of infection by a pathogen, or any detectable reduction in the severity of the disease or any symptom or condition resulting from infection by the pathogen in the vaccinated animal as compared to an unvaccinated infected animal. An effective immunoprotective response can be induced in animals that have not previously been infected with the pathogen and/or are not infected with the pathogen at the time of vaccination. An effective immunoprotective response can also be induced in an animal already infected with the pathogen at the time of vaccination.

[0051] An "antigenic epitope" is, unless otherwise indicated, a molecule that is able to elicit an immune response in a particular animal or species. Antigenic epitopes are proteinaceous molecules, *i.e.* polyaminoacid sequences, optionally comprising non-protein groups such as carbohydrate moieties and/or lipid moieties.

[0052] The term "pathogenically infecting" used herein refers to the ability of a pathogen to infect an animal and cause a disease in the animal. As an example, a PRRS virus is capable of pathogenically infecting a porcine animal since it can cause PRRS in swine. However, although a PRRS virus may be able to infect, either productively or non-productively, a bird or another mammal, such as a human, it does not pathogenically infect any animal other than a porcine animal since it does not cause any disease in animals other than porcine animals.

[0053] The genetically modified North American PRRS viruses encoded by the above-described isolated polynucleotide molecules are, in one embodiment, able to elicit an effective immunoprotective response against infection by a PRRS virus. Such genetically modified North American PRRS viruses are preferably able to elicit an effective immunoprotective response against any strain of PRRS viruses, including both European and North American strains.

[0054] In one embodiment, the mutation or mutations in the isolated polynucleotide molecule encoding the genetically disabled North American PRRS virus are non-silent and occur in one or more open reading frames of the nucleotide sequence encoding the North American PRRS virus; *i.e.*, the mutation or mutations occur in one or more of the sequences within the nucleotide sequence encoding the North American PRRS virus that are the same as ORFs 1a, 1b, 2, 3, 4, 5, 6, or 7 of SEQ ID NO:1. In another embodiment, the mutation or mutations occur in one or more noncoding regions of the North American PRRS virus genome, such as, for example, in the leader sequence of the North American PRRS virus genome; *i.e.*, the mutation or mutations occur within the sequence that is the same as the sequence of nucleotides 1-191 of SEQ ID NO:1. In the same isolated polynucleotide molecule, mutations can occur in both coding and noncoding regions.

[0055] As used herein, unless otherwise indicated, "noncoding regions" of the nucleotide sequence encoding the North American PRRS virus refer to those sequences of RNA that are not translated into a protein and those sequences of cDNA that encode such RNA sequences. Coding regions refer to those sequences of RNA from which North American PRRS virus proteins are expressed, and also refer to cDNA that encodes such RNA sequences. Likewise, "ORFs" refer both to RNA sequences that encode North American PRRS virus proteins and to cDNA sequence encoding such RNA sequences.

[0056] Determining suitable locations for a mutation or mutations that will encode a North American PRRS virus that is genetically disabled so that it is unable to produce PRRS yet remains able to elicit an effective immunoprotective response against infection by a PRRS virus can be made based on the SEQ ID NO:1 provided herein. One of ordinary skill can refer to the sequence of the infectious cDNA done of North American PRRS virus provided by this invention, make sequence changes which will result in a mutation, and test the viruses encoded thereby both for their ability to produce PRRS in swine, and to elicit an effective immunoprotective response against infection by a PRRS virus. In so doing, one of ordinary skill can refer to techniques known in the art and also those described and/or exemplified herein.

[0057] For example, an ORF of the sequence encoding the infectious RNA molecule encoding the North American PRRS virus can be mutated and the resulting genetically modified North American PRRS virus tested for its ability to cause PRRS. The ORF of a North American PRRS virus encodes proteins as follows: ORF 1a encodes a polyprotein comprising protease function; ORF 1b encodes a polyprotein comprising replicase (RNA polymerase) and helicase functions; ORFs 2, 3, and 4 encode small membrane glycoproteins; ORF 5 encodes a major envelope glycoprotein; ORF 6 encodes a nonglycosylated integral membrane protein; and ORF 7 encodes a nucleocapsid protein. Genetic mutations of one or more of these ORFs can be used in preparing the genetically modified North American PRRS viruses described *infra*.

[0058] The subject invention also provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more heterologous antigenic epitopes, wherein the DNA sequence encoding the RNA molecule encoding the North American PRRS virus is SEQ ID NO:1 and further comprising one or more additional nucleotide sequences that each encode a heterologous antigenic epitope, and wherein each heterologous antigenic epitope is capable of inducing an effective immunoprotective response against a particular pathogen in a mammal or a bird.

[0059] A pathogen against which an effective immunoprotective response can be induced by means of the above recited aspect of the present invention is any pathogen, such as a virus, bacteria, fungus, or protozoan, capable of causing a disease in a mammal or bird, which pathogen comprises or has associated therewith one or more antigenic epitopes which can be used to induce an effective immunoprotective response against the pathogen in the mammal or bird.

[0060] The term "heterologous antigenic epitope" for purposes of the present invention means an antigenic epitope, as defined above, not normally found in a wild-type North American PRRS virus. A nucleotide sequence encoding a heterologous antigenic epitope can be inserted into a North American PRRS viral genome using known recombinant techniques. Antigenic epitopes useful as heterologous antigenic epitopes for the present invention include additional North American PRRS virus antigenic epitopes, antigenic epitopes from European PRRS viruses, antigenic epitopes from swine pathogens other than PRRS viruses, or antigenic epitopes from pathogens that pathogenically infect birds or mammals other than swine, including humans. Sequences encoding such antigenic epitopes are known in the art or are provided herein. For example, a second North American PRRS virus envelope protein, encoded by North American PRRS ORF 5 described herein, can be inserted into a DNA sequence encoding an RNA molecule encoding a North American PRRS virus of the present invention to generate a genetically modified North American PRRS virus comprising an additional envelope protein as a heterologous antigenic epitope. Such a genetically modified North American PRRS virus can be used to induce a more effective immunoprotective response against PRRS viruses in a porcine animal vaccinated therewith.

[0061] Examples of an antigenic epitope from a swine pathogen other than a North American PRRS virus include, but are not limited to, an antigenic epitope from a swine pathogen selected from the group consisting of European PRRS, porcine parvovirus, porcine circovirus, a porcine rotavirus, swine influenza, pseudorabies virus, transmissible gastroenteritis virus, porcine respiratory coronavirus, classical swine fever virus, African swine fever virus, encephalomyocarditis virus, porcine paramyxovirus, *Actinobacillus pleuropneumoni*, *Bacillus anthraci*, *Bordetella bronchiseptica*, *Clostridium*

haemolyticum, *Clostridium perfringens*, *Clostridium tetani*, *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Haemophilus parasuis*, *Leptospira spp.*, *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis*, *Pasteurella haemolytica*, *Pasteurella multocida*, *Salmonella choleraesuis*, *Salmonella typhimurium*, *Streptococcus equisimilis*, and *Streptococcus suis*. Nucleotide sequences encoding antigenic epitopes from the aforementioned swine pathogens are known in the art and can be obtained from public gene databases such as GenBank (<http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html>) provided by NCBI.

[0062] If the heterologous antigenic epitopes are antigenic epitopes from one or more other swine pathogens, then the isolated polynucleotide molecule can further contain one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS. Such isolated polynucleotide molecules and the viruses they encode are useful for preparing vaccines for protecting swine against the swine pathogen or pathogens from which the heterologous antigenic epitopes are derived.

[0063] In a preferred embodiment, the genetically modified North American PRRS is able to elicit an effective immunoprotective response against infection by a PRRS virus in a porcine animal. Such isolated polynucleotide molecules and the viruses they encode are useful for preparing dual-function vaccines for protecting swine against infection by both a North American PRRS virus and the swine pathogen or pathogens from which the heterologous antigenic epitopes are derived. In another preferred embodiment, the genetically modified North American PRRS virus useful in a dual-function vaccine is genetically disabled.

[0064] The isolated polynucleotide molecules of the present invention comprising nucleotide sequences encoding heterologous antigenic epitopes can be prepared as described above based on the sequence encoding a North American PRRS virus described herein using known techniques in molecular biology.

[0065] In a further preferred embodiment, a heterologous antigenic epitope of the genetically modified North American PRRS virus of the present invention is a detectable antigenic epitope. Such isolated polynucleotide molecules and the North American PRRS viruses they encode are useful, *inter alia*, for studying PRRS infections in swine, determining successfully vaccinated swine, and/or for distinguishing vaccinated swine from swine infected by a wild-type PRRS virus. Preferably, such isolated polynucleotide molecules further contain one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS, and more preferably are able to elicit an effective immunoprotective response in a porcine animal against infection by a PRRS virus.

[0066] Heterologous antigenic epitopes that are detectable, and the sequences that encode them, are known in the art. Techniques for detecting such antigenic epitopes are also known in the art and include serological detection of antibody specific to the heterologous antigenic epitope by means of, for example, Western blot, ELISA, or fluorescently labeled antibodies capable of binding to the antibodies specific to the heterologous antigenic epitope. Techniques for serological detection useful in practicing the present invention can be found in texts recognized in the art, such as Coligan, J.E., *et al.* (eds), 1998, *Current Protocols in Immunology*, John Wiley & Sons, Inc. Alternatively, the heterologous antigenic epitope itself can be detected by, for example, contacting samples that potentially comprise the antigenic epitope with fluorescently-labeled antibodies or radioactively-labeled antibodies that specifically bind to the antigenic epitopes.

[0067] The present invention further provides an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule which encodes a genetically modified North American PRRS virus that detectably lacks a North American PRRS virus antigenic epitope, wherein the DNA sequence encoding the RNA molecule encoding the North American PRRS virus is SEQ ID NO:1 except that it lacks one or more nucleotide sequences encoding a detectable North American PRRS virus antigenic epitope. Such isolated polynucleotide molecules are useful for distinguishing between swine infected with a recombinant North American PRRS virus of the present invention and swine infected with a wild-type PRRS virus. For example, animals vaccinated with killed, live or attenuated North American PRRS virus encoded by such an isolated polynucleotide molecule can be distinguished from animals infected with wild-type PRRS based on the absence of antibodies specific to the missing antigenic epitope, or based on the absence of the antigenic epitope itself. If antibodies specific to the missing antigenic epitope, or if the antigenic epitope itself, are detected in the animal, then the animal was exposed to and infected by a wild-type PRRS virus. Means for detecting antigenic epitopes and antibodies specific thereto are known in the art, as discussed above. Preferably, such an isolated polynucleotide molecule further contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS. More preferably, the encoded virus remains able to elicit an effective immunoprotective response against infection by a PRRS virus.

B. Plasmids Encoding a North American PRRS Virus or a Genetically Modified North American PRRS Virus:

[0068] The present invention also provides any of the above-described isolated polynucleotide molecules in the form of a plasmid capable of expressing the North American PRRS virus encoded thereby.

[0069] Plasmids of the present invention can express the encoded North American PRRS virus outside of a living organism, to produce North American PRRS viruses of the invention useful, *inter alia*, for preparing vaccines. In one

embodiment, a plasmid of the present invention capable of expressing a North American PRRS virus outside of a living organism is a plasmid wherein transcription of viral RNA therefrom occurs *in vitro* (i.e. extracellularly); the resulting viral RNA molecule is transfected into a suitable host cell using known mechanisms of transfection, such as electroporation, lipofection (in some cases using a commercially available reagent, such as Lipofectin™ (Life Technologies Inc., Rockville, Maryland, USA)), or DEAE dextran mediated transfection. Other methods of transfection are known in the art and can be employed in the present invention. An example of such a plasmid for *in vitro* transcription of North American PRRS viral RNA is the plasmid pT7P129A (ATCC Accession No. 203488). Any promoter useful for *in vitro* transcription can be used in such plasmids of this invention. T7 is one such promoter, but other promoters can be used, such as an SP6 promoter or a T3 promoter. The sequences of such promoters can be artificially synthesized or cloned from commercially available plasmids. Suitable plasmids for preparing such plasmids capable of expressing North American PRRS virus include, but are not limited to, general purpose cloning vector plasmids such as pCR2.1 (Invitrogen, Carlsbad, California, USA), pBR322, and pUC18. A nucleotide sequence of the present invention encoding the North American PRRS virus can be inserted into any of these plasmids using known recombinant techniques. Other plasmids into which the polynucleotide molecules of the present invention can be inserted will be recognized by those of ordinary skill in the art.

[0070] Suitable conditions for *in vitro* transcription of viral RNA from any of the above-described recombinant plasmids comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus depends on the type of plasmid, for example, its particular promoter, and can be ascertained by one of ordinary skill in the art. For example, if a plasmid of the present invention is based on a pCR2.1 plasmid comprising a T7 promoter, then an example of suitable conditions for *in vitro* transcription includes reacting the plasmid with T7 RNA polymerase and ribonucleotides in a standard buffer and incubating the reaction at 37°C for about 30 minutes. In some cases, commercial kits are available for transcribing RNA from a particular plasmid, and such kits can be used in the present invention. The reaction mixture following transcription can be directly transfected into a suitable host cell without purification, or the transcribed North American PRRS virus RNA can be purified by known RNA purification techniques, for example by organic (e.g. phenol) extraction and alcohol (e.g. ethanol or isopropanol) precipitation, prior to transfection.

[0071] Practically any mammalian or avian cell culture can be transfected with the North American PRRS virus RNA obtained as described above in order to generate a first round of North American PRRS virions. An example of cells which one might find particularly useful because of their ready availability and ease of use are BHK (baby hamster kidney) cells. However, if one wishes to generate a cell culture capable of sustained production of North American PRRS virions, then porcine alveolar macrophage cells or MARC-145 cells (Kim, H.S., *et al.*, *supra*) are preferred since these cells excrete high levels of new generation PRRS virions subsequent to PRRS virus infection. Other cell lines derived from the MA-104 cell line may also be used for sustained generation of North American PRRS virions of the present invention. Primary porcine alveolar macrophage cells can be obtained by lung lavages from pigs, and the MARC-145 monkey kidney cell line can be obtained from the National Veterinary Services Laboratories otherwise known as NVSL (Ames, Iowa, USA).

[0072] In another embodiment, a plasmid capable of expressing a North American PRRS virus of the present invention outside of a living organism is a plasmid which is transfected into a suitable host cell, for example by electroporation or lipofection, transcription of the infectious RNA molecule and expression of the North American PRRS virus therefrom occurring within the transfected host cell. The transfected host cell therefore generates North American PRRS virions. Such a completely cellular method has heretofore never been disclosed or suggested for any virus within the order of *Nidovirales*. Because of possible cryptic splicing and termination sequences present in the RNA genome of viruses of the *Nidovirales* order, a completely cellular method of expressing a *Nidovirales* virus was believed unlikely. Cryptic sequences include RNA splice donor and splice acceptor sequences, which could cause inappropriate splicing of the RNA transcript, as well as polyadenylation sequences, which could cause premature termination by the cellular RNA polymerase II. The present invention demonstrates, however, that the presence of such sequences in a plasmid comprising a cDNA clone of a Nidovirus does not prevent the plasmid's ability to express the Nidovirus when the plasmid is directly transfected into a suitable host cell.

[0073] Suitable plasmids that can be used to prepare recombinant plasmids of the present invention for completely cellular expression outside of a living organism of a *Nidovirales* virus, such as a PRRS virus, include virtually any plasmid useful for transfection and expression in eukaryotic cells. An examples of a plasmid suitable for preparing recombinant plasmids of the present invention for completely cellular expression of a *Nidovirales* virus is the plasmid pCMVbeta (Clontech, Palo Alto, California, USA). Other plasmids which are able to transfect and express genes in eukaryotic cells which can be used to prepare plasmids of the present invention include, but are not limited to, pcDNA3.1, pRc/RSV, and pZeoSV2 (all from Invitrogen); and pCMV-Sport3 and pSV-Sport1 (both from Life Technologies Inc.). However, almost any eukaryotic expression vector will work for the present invention. Constructs based on cosmids can also be used for completely cellular *ex vivo* expression of a *Nidovirales* virus.

[0074] Suitable host cells for the completely cellular method of the present invention for expressing PRRS virus include porcine alveolar macrophage cells and the MARC-145 cells, described above. Methods of transfecting these cells with a plasmid are basically the same as those methods for transfecting cells with viral RNA described above. Such methods

include, but are not limited to, electroporation, lipofection, DEAE dextran mediated transfection, and calcium phosphate coprecipitation.

[0075] Once host cells, such as porcine alveolar macrophage cells or a MARC-145 cells, have been transfected according to the subject invention, either with viral RNA or with a plasmid comprising a nucleotide sequence encoding a virus, then the cells can be frozen at about -80°C or below for storage for up to several years. For longer periods of time, *i.e.* decades, storage in liquid nitrogen is preferred. If relatively frequent use of the encoded virus is envisioned, then cells hosting the virus can also be maintained (unfrozen) in culture using known techniques, for shorter periods of time. Moreover, viral particles excreted by such cells can be stored frozen at about -80°C or below as a source of virus. Transfection of such cell lines with the polynucleotide molecule encoding the virus can be confirmed if desired, for example, by testing exhausted medium excreted by the cell line for a PRRS virus antigen using an immunofluorescent antibody test. Antibodies which are specific for PRRS virus antigens are known in the art (see, *e.g.*, Collins, E.J., *et al.*, WO 93/03760 March 4, 1993).

[0076] In another embodiment, a plasmid of the present invention comprising a nucleotide sequence encoding a North American PRRS virus is suitable for *in vivo* expression of the North American PRRS virus, *i.e.* expression in a living organism. Plasmids which can be used for preparing recombinant plasmids for *in vivo* expression of a North American PRRS virus include, but are not limited to the plasmids capable of transfecting eukaryotic cells described above, such as pCMVbeta.

[0077] Animals that can be transfected with plasmids of the present invention include mammals and birds. If the animal is other than a porcine animal, for example, a mallard duck, then the plasmid can comprise a nucleotide sequence encoding a North American PRRS virus comprising further antigenic epitopes from pathogens which are capable of pathogenically infecting the animal; in such a case, the plasmid will encode a North American PRRS virus serving as a vector for transporting epitopes into the animal. If the animal is a porcine animal, then the plasmid can usefully encode any of the North American PRRS viruses described herein, including the genetically-modified North American PRRS viruses described herein.

C. Viral Vectors Encoding a North American PRRS Virus, Including Viral Vectors Encoding Genetically Modified North American PRRS Viruses:

[0078] The present invention also provides viral vectors comprising a DNA sequence encoding an infectious RNA molecule encoding any of the North American PRRS viruses described herein, including the genetically-modified North American PRRS viruses described herein. Such viral vectors are useful for transfecting eukaryotic cells for production of PRRS viruses of the present invention outside of a living organism, or for transfecting swine, or other mammals, or avians, with the sequence encoding the North American PRRS virus, for *in vivo* expression of the North American PRRS virus therein.

[0079] Some examples of viruses that can be used as vectors for preparing the viral vectors of the present invention include, but are not limited to, swine viruses such as, but not limited to, swine pox virus, pseudorabies virus, or African swine fever virus. Such swine viruses can be obtained from The National Veterinary Services Laboratories (Ames, Iowa, USA) of the United States Department of Agriculture; the American Type Culture Collection, otherwise known as the ATCC (Manassas, Virginia, USA); and other known sources. Recombinant viral vectors based on suitable swine viruses such as the aforementioned swine viruses are useful for transfecting swine animals with a nucleotide sequence encoding a North American PRRS virus of the present invention.

[0080] Viral vectors comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus of the present invention based on these and other viruses can be prepared using known recombinant techniques described in texts such as those cited previously in this application.

D. Transfected Host cells Encoding or a Genetically Modified North American PRRS Viruses:

[0081] The present invention also provides transfected host cells that comprise a DNA sequence encoding an infectious RNA molecule encoding any of the North American PRRS viruses described herein, including the genetically-modified North American PRRS viruses described herein, which transfected host cells are capable of expressing the North American PRRS virus. Such transfected host cells are useful for producing North American PRRS viruses of the present invention. Examples of transfected host cells of the present invention include the transfected porcine alveolar macrophage cells and the transfected MARC-145 cells described above.

[0082] Other transfected host cells of the invention include, but are not limited to, transfected MA-104 cells and other derivatives of MA-104 cells that are transfected; transfected Baby Hamster Kidney (BHK) cells; transfected Chinese Hamster Ovary (CHO) cells; and African Green Monkey kidney cells other than MA-104 cells or MARC-145 cells, such as VERO cells; that are transfected.

E. North American PRRS Viruses, Including Genetically Modified North American PRRS Viruses:

[0083] The present invention also provides North American PRRS viruses as described herein, including genetically-modified North American PRRS viruses as described herein, expressed and/or encoded by any of the above-described isolated polynucleotide molecules, RNA molecules, plasmids, viral vectors, or transfected host cells.

[0084] In certain situations, for example where the North American PRRS virus is to be used in a vaccine for swine and the North American PRRS virus has not been genetically modified as described above so as to be unable to cause PRRS, it is desirable to treat the North American PRRS virus, for example by inactivating or attenuating it, so that it is unable to cause PRRS in swine to which it is administered. Known methods can be used to inactivate a North American PRRS virus of the present invention so that it is unable to cause PRRS in an animal. Examples of such methods include, but are not limited to, treatment with formaldehyde, BEI (binary ethyleneimine), or BPL (beta-propiolactone). Methods of attenuation are also known in the art, and such methods can be used to attenuate a North American PRRS virus of the present invention. A North American PRRS virus of the present invention can, for example, be attenuated by serial passage in cell culture.

[0085] If a North American PRRS virus of the present invention is for use in an animal other than a porcine animal, or if it has been genetically modified as described herein so that it is unable to produce PRRS in a porcine animal, then it is not necessary to treat the virus as described in the preceding paragraph prior to using it in a vaccine.

F. Vaccines and Uses Thereof:

[0086] The present invention also provides vaccines comprising North American PRRS viruses, including genetically modified North American PRRS viruses disabled in their ability to produce PRRS in a swine animal as described herein; infectious RNA molecules and plasmids encoding such North American PRRS viruses as described herein; and viral vectors encoding such North American PRRS viruses and isolated RNA molecules as described herein. The invention also provides methods for protecting animals from infection comprising vaccination with such vaccines.

[0087] In a preferred embodiment, the subject invention provides a vaccine comprising a genetically modified North American PRRS virus comprising one or more heterologous antigenic epitopes as described herein, an infectious RNA molecule encoding such a genetically modified North American PRRS virus, or a plasmid as described herein encoding such a genetically modified North American PRRS virus, in an amount effective to elicit an immunoprotective response against infection by the pathogen or pathogens from which the heterologous antigenic epitope(s) are derived, and a carrier acceptable for pharmaceutical or veterinary use.

[0088] Such vaccines can be used to protect from infection a mammal or a bird capable of being pathogenically infected by the pathogen or pathogens from which the heterologous antigenic epitope(s) are derived. Accordingly, the subject invention also provides a method for protecting a mammal or a bird from infection by a pathogen, which comprises vaccinating the mammal or bird with an amount of the vaccine described in the preceding paragraph effective to elicit an immunoprotective response in the mammal or bird from infection by the pathogen.

[0089] In a further preferred embodiment, the vaccine comprises a genetically modified North American PRRS virus, or an infectious RNA molecule or plasmid encoding such a genetically modified North American PRRS virus, comprising or encoding one or more heterologous antigenic epitopes from a swine pathogen other than a North American PRRS virus. These vaccines are useful for protecting swine from infection by the swine pathogen or pathogens from which the heterologous antigenic epitope(s) are derived. If such a vaccine comprises the genetically modified North American PRRS virus, the genetic modification of the North American PRRS virus preferably renders the virus unable to cause PRRS in swine. In another preferred embodiment, the genetically modified North American PRRS virus in the vaccine is able to elicit an immunoprotective response against infection by a PRRS virus, thus providing a dual-vaccine for swine, protecting swine from infection by the swine pathogen or pathogens from which the heterologous antigenic epitope(s) are derived as well as from infection by a PRRS virus. If the vaccine comprises an infectious RNA molecule or a plasmid encoding a genetically-modified North American PRRS virus comprising one or more heterologous antigenic epitopes from another swine pathogen, then the sequence encoding the infectious RNA molecule encoding the genetically modified PRRS virus preferably comprises one or more further mutations that genetically disable the encoded North American PRRS virus so that it is unable to cause PRRS. In another preferred embodiment, the encoded genetically modified, disabled North American PRRS virus is able to elicit an immunoprotective response against a PRRS infection in a swine animal, thus providing a dual-vaccine for swine, able to protect swine from infection by the swine pathogen or pathogens from which the heterologous antigenic epitope(s) are derived as well as from infection by a PRRS virus. All of these vaccines also further comprise a carrier acceptable for veterinary use.

[0090] Vaccines of the present invention can be formulated following accepted convention to include 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.

[0091] Vaccines of the present invention can further comprise one or more additional immunomodulatory components such as, e.g., an adjuvant or cytokine, among others. Nonlimiting examples of adjuvants that can be used in the vaccine of the present invention include the RIBI adjuvant system (Ribi Inc., Hamilton, MT), alum, mineral gels such as aluminum hydroxide gel, oil-in-water emulsions, water-in-oil emulsions such as, e.g., Freund's complete and incomplete adjuvants, Block copolymer (CytRx, Atlanta GA), QS-21 (Cambridge Biotech Inc., Cambridge MA), SAF-M (Chiron, Emeryville CA), AMPHIGEN® adjuvant, saponin, Quil A or other saponin fraction, monophosphoryl lipid A, and Avridine lipid-amine adjuvant. Nonlimiting 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. Other immunomodulatory agents that can be included in the vaccine include, e.g., one or more interleukins, interferons, or other known cytokines.

[0092] Vaccines of the present invention can optionally be formulated for sustained release of the virus, infectious RNA molecule, plasmid, or viral vector of the present invention. Examples of such sustained release formulations include virus, infectious RNA molecule, plasmid, or viral vector in combination with composites of biocompatible polymers, such as, e.g., poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including A. Domb *et al.*, 1992, Polymers for Advanced Technologies 3: 279-292, which is incorporated herein by reference. Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in texts known in the art, for example M. Chasin and R. Langer (eds), 1990, "Biodegradable Polymers as Drug Delivery Systems" in: Drugs and the Pharmaceutical Sciences, Vol. 45, M. Dekker, NY. Alternatively, or additionally, the virus, plasmid, or viral vector can be microencapsulated to improve administration and efficacy. Methods for microencapsulating antigens are well-known in the art, and include techniques described, e.g., in U.S. Patent 3,137,631; U.S. Patent 3,959,457; U.S. Patent 4,205,060; U.S. Patent 4,606,940; U.S. Patent 4,744,933; U.S. Patent 5,132,117; and International Patent Publication WO 95/28227.

[0093] Liposomes can also be used to provide for the sustained release of virus, plasmid, or viral vector. Details concerning how to make and use liposomal formulations can be found in, among other places, U.S. Patent 4,016,100; U.S. Patent 4,452,747; U.S. Patent 4,921,706; U.S. Patent 4,927,637; U.S. Patent 4,944,948; U.S. Patent 5,008,050; and U.S. Patent 5,009,956.

[0094] An effective amount of any of the above-described vaccines can be determined by conventional means, starting with a low dose of virus, plasmid or viral vector, and then increasing the dosage while monitoring the effects. An effective amount may be obtained after a single administration of a vaccine or after multiple administrations of a vaccine. Known factors can be taken into consideration when determining an optimal dose per animal. These include the species, size, age and general condition of the animal, the presence of other drugs in the animal, and the like. The actual dosage is preferably chosen after consideration of the results from other animal studies.

[0095] One method of detecting whether an adequate immune response has been achieved is to determine seroconversion and antibody titer in the animal after vaccination. The timing of vaccination and the number of boosters, if any, will preferably be determined by a doctor or veterinarian based on analysis of all relevant factors, some of which are described above.

[0096] The effective dose amount of virus, infectious RNA molecule, plasmid, or viral vector, of the present invention can be determined using known techniques, taking into account factors that can be determined by one of ordinary skill in the art such as the weight of the animal to be vaccinated. The dose amount of virus of the present invention in a vaccine of the present invention preferably ranges from about 10^1 to about 10^9 pfu (plaque forming units), more preferably from about 10^2 to about 10^8 pfu, and most preferably from about 10^3 to about 10^7 pfu. The dose amount of a plasmid of the present invention in a vaccine of the present invention preferably ranges from about 0.1 µg to about 100mg, more preferably from about 1µg to about 10mg, even more preferably from about 10µg to about 1mg. The dose amount of an infectious RNA molecule of the present invention in a vaccine of the present invention preferably ranges from about 0.1µg to about 100mg, more preferably from about 1 µg to about 10mg, even more preferably from about 10µg to about 1mg. The dose amount of a viral vector of the present invention in a vaccine of the present invention preferably ranges from about 10^1 pfu to about 10^9 pfu, more preferably from about 10^2 pfu to about 10^8 pfu, and even more preferably from about 10^3 to about 10^7 pfu. A suitable dosage size ranges from about 0.5 ml to about 10 ml, and more preferably from about 1 ml to about 5 ml.

[0097] The present invention further provides a method of preparing a vaccine comprising a North American PRRS virus, infectious RNA molecule, plasmid, or viral vector described herein, which method comprises combining an effective

amount of one of the North American PRRS virus, infectious RNA molecule, plasmid, or viral vector of the present invention, with a carrier acceptable for pharmaceutical or veterinary use.

[0098] It is to be understood that the term "North American PRRS viruses of the present invention" and like terms, unless otherwise indicated, include any of the genetically modified North American PRRS viruses described herein as well as the unmodified North American PRRS virus described herein encoded by SEQ ID NO:1.

G. Isolated Polynucleotide Molecules and Transfected Host cells Encoding North American PRRS Virus Peptides, and Methods for Making Functional North American PRRS Virions:

[0099] The present invention also provides an isolated polynucleotide molecule comprising one or more nucleotide sequences that encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA molecule corresponding to SEQ ID NO:1. As used herein, terms such as "North American PRRS virus peptide" mean a peptide that is expressed by a North American PRRS virus. Such a peptide can be, but is not necessarily, specific to North American PRRS viruses.

[0100] In a preferred embodiment, an isolated polynucleotide molecule of the present invention encoding a North American PRRS virus peptide comprises a sequence or sequences independently selected from SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9. Such isolated polynucleotide molecules are useful in plasmids or for preparing viral vectors for transfecting a suitable host cell to create a "helper" cell comprising one or more nucleotide sequences that encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA sequence corresponding to SEQ ID NO:1, which helper cell is useful in the preparation of functional virions of genetically modified North American PRRS viruses of the present invention, which viruses have been genetically modified as described above so that they are missing from their RNA genome the sequence(s) encoding the peptide or peptides encoded by the helper cell.

[0101] Accordingly, the subject invention also includes plasmids and viral vectors comprising one or more nucleotide sequences encoding a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA sequence corresponding to SEQ ID NO:1. Such plasmids of the invention can be based on those plasmids described above useful for preparing plasmids comprising a nucleotide sequence encoding a North American PRRS virus. Such viral vectors of the invention can be based on, for example, retrovirus vectors or adeno-associated viral vectors. These plasmids and viral vectors are useful for preparing the helper cells described in the preceding paragraph.

[0102] The present invention also thus provides a helper cell, *i.e.*, a transfected host cell comprising one or more nucleotide sequences that encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA sequence corresponding to SEQ ID NO:1. In preferred embodiments, the transfected host cell comprises a nucleotide sequence or sequences independently selected from SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9. These helper cells are useful as described above for providing peptides for completing infectious RNA molecules deficient in sequences encoding the peptide(s) encoded by the helper cell so that functional virions of a genetically modified North American PRRS virus can be generated by the cell.

[0103] Suitable cells for this aspect of the invention include the cells described above which are suitable for expressing North American PRRS viruses, such as the porcine alveolar macrophage cells and the MARC-145 cells. However, practically any mammalian or avian cell can be used. As discussed above, if one wishes to obtain a transfected host cell capable of reinfection by PRRS virions, and thus able to generate multiple generations of functional genetically modified North American PRRS virions, porcine alveolar macrophage cells and MARC-145 cells are preferred.

[0104] The subject invention thus further provides a method for generating a functional virion of a genetically modified North American PRRS virus encoded by an RNA sequence corresponding to SEQ ID NO:1 comprising one or more mutations that disable one or more peptide coding sequences, which method comprises transfecting a helper cell as described in the preceding paragraph with an infectious RNA molecule, plasmid, or viral vector encoding the genetically modified North American PRRS virus, which helper cell comprises a nucleotide sequence or sequences encoding the North American PRRS virus peptide or peptides of the disabled peptide coding sequence(s) of the genetically modified North American PRRS virus.

[0105] Infectious RNA molecules, plasmids, and viral vectors that encode a genetically modified North American PRRS virus useful in either of the above methods for generating a functional virion of a genetically modified North American PRRS virus are those infectious RNA molecules, plasmids, and viral vectors described in this application.

Examples**Example I. Preparation of an Infectious cDNA Clone of a North American PRRS Virus Isolate.**

[0106] Source of PRRS virus and MARC-145 cells: A North American PRRS virus isolate designated P129 was obtained from Drs. Gregory W. Stevenson, William G. Van Alstine, and Charles L. Kanitz of Purdue University's Animal Disease Diagnostic Laboratory in West Lafayette, Indiana. The P129 virus was originally isolated in the autumn of 1995 from a swine herd in southern Indiana experiencing a severe PRRS outbreak. This farm had no previous history of PRRS problems or PRRS vaccination. The P129 isolate was more virulent than several other field isolates from the same time period and geographic area, in that it produced more severe and more consistent respiratory disease in young pigs. The virus was initially isolated on primary porcine alveolar macrophage (the natural host cell), and subsequently passaged on MARC-145 cells (Kim, H.S. *et al.*, 1993, Arch. Virol. 133:477-483). Genes encoding structural proteins of P129 were found to be homologous to corresponding known North American PRRS gene sequences.

[0107] The MARC-145 cell line that was used to propagate PRRS viruses is a clone of the MA-104 Rhesus Macaque Monkey Kidney cell line. The MARC-145 cells were obtained from the National Veterinary Services Laboratories (NVSL, Ames, Iowa) of the USDA. These cells have been tested and found negative for mycoplasmas and for common porcine extraneous agents. MARC-145 cells are routinely grown at 37°C in OptiMEM (Life Technologies Inc.) with 2% fetal bovine serum and antibiotics.

[0108] Five biological clones were plaque purified from the P129 virus stock, and these were designated P129A through P129E. Plaque purification was carried out by infecting monolayers of MARC-145 cells with P129 virus, adding an overlay of OptiMEM containing 1.25% SeaPlaque agarose (FMC BioProducts), 2% fetal bovine serum, and antibiotics. Plaques were clearly visible following incubation for 7 days, when 5 well-isolated plaques were picked and passaged onto fresh MARC-145 monolayers. When cytopathic effect (virus induced cell death) became apparent the progeny virus from each of these cultures was subjected to another round of plaque purification. One well-isolated plaque from each of the five clones was picked and expanded to produce large stocks. The 5 clones were tested for virulence in young pigs, either individually (clones A and E) or in combination (clones B-D, or clones A-E). In all cases, the plaque purified virus replicated well in pigs and caused clinical disease. The severity of clinical symptoms was less than that caused by the uncloned P129 virus, even when all five clones were used together. P129A was chosen for sequencing, and was used in subsequent molecular manipulations.

[0109] Determination of the genome sequence of P129A: Plaque purified virus P129A was used for sequence determination after 10 serial passages from the pig (including two plaque purifications and one subsequent passage). SEQ ID NO:1 shows the cDNA sequence corresponding to the P129A RNA genome. The genome is 15,395 nucleotides in length (excluding the polyadenosine tail), begins with ATGACGTA, and ends with CCGCAATT. A typical polyadenosine tail of 55 residues is also provided in SEQ ID NO:1.

[0110] For the structural genes of P129A (ORFs 2 through 7), which comprise the 3' 20% of the genome, various PCR primers were chosen based on several partial cDNA sequences of other North American PRRS virus isolates available in the public DNA sequence database GenBank (for example PRU00153). Purified viral RNA was reverse transcribed into cDNA using reverse transcriptase and random hexamer primers. This cDNA was then used in PCR with gene-specific primers. PCR products were excised from gels and T/A cloned into plasmid pCR2.1 (Invitrogen). For each primer pair, multiple plasmids (from independent PCR reactions) were DNA sequenced. Sequences were assembled using the Seqman program from the Lasergene package (DNASTAR, Inc). This permitted completing the sequence of positions 11,992 through 15,347 of the P129A genome.

[0111] Also in the GenBank database are a series of short sequences (approximately 218 nucleotides total) which comprise a portion of the ORF 1b gene of several isolates of PRRS virus. One of these (PPSSEQB) was used to design PCR primers (forward 5'-ACAGTTTGGTGATCTATG-3' (SEQ ID NO:10), corresponding to positions 9063-9080; reverse 5'-CAGATTCAGATGTTCAA-3' (SEQ ID NO:11), corresponding to positions 9252-9268). These amplified a 206 nucleotide fragments, which includes 171 nucleotides of new sequence from the P129A ORF1b gene, corresponds to positions 9081 to 9251. A new forward primer was designed within this region (5'-ACCTCGTGCTGTATGCCGAATCTC-3' (SEQ ID NO:12), positions 9201-9224), and a matching primer was designed within ORF1b immediately upstream of ORF2 (5'-TCAGGCCTAAAGTTGGTTCAATGA-3' (SEQ ID NO:13), positions 12,027-12,050). These primers were used in RT-PCR to amplify a 2850 nucleotide fragment of ORF1b, corresponding to positions 9201-12,050 of the P129A genome.

[0112] During RT-PCR amplification of ORF5 of another North American field isolate of PRRS virus, a minor band was seen which was smaller than the expected size. This was sequenced and found to have limited homology with ORF1a of Lelystad virus (resulting from false priming). New primers within this region were chosen to amplify P129A (forward 5'-GATGACTGGGCTACTGACGAGGAT-3' (SEQ ID NO:14), corresponding to positions 1587-1610; reverse 5'-AGAGCGGCTGGGATGACACTG-3' (SEQ ID NO:15), corresponding to positions 1877-1897). In addition to the product of 311 nucleotides (266 nucleotides of new P129A sequence between the primers corresponding to positions 1611-1876), a larger minor PCR product of 701 nucleotides was cloned and sequenced (656 nucleotides of new P129A

sequence between the primers corresponding to positions 1611-2264). The larger band results from false priming of the reverse primer at positions 2265-2269.

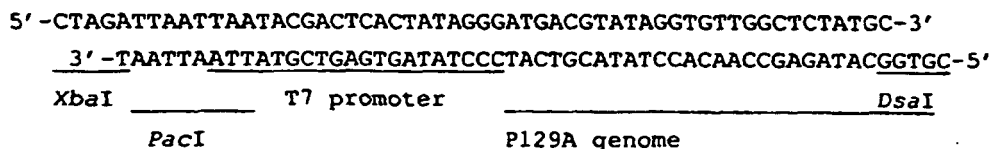
[0113] The extreme 5' end of the genome of P129A was determined by 5' RACE (rapid amplification of cDNA ends) using a commercially available kit (Life Technologies Inc). Two nested reverse primers were chosen from within the known ORF1a sequence ("RACE2" 5'-CCGGGGAAGCCAGACGATTGAA-3' (SEQ ID NO:16), positions 1917-1938; and "RACE3" 5'-AGGGGGAGCAAAGAAGGGGTCATC-3' (SEQ ID NO:17), positions 1733-1756). RACE2 was used to prime cDNA synthesis, while RACE3 was used in PCR. The resulting PCR products were cloned and sequenced. The two longest products ended at precisely the same base (position 1 in SEQ ID NO:1).

[0114] The large gap between known sequence in ORF1a and ORF1b was bridged using long RT-PCR. Two new primers were used (forward 5'- AGCACGCTCTGGTCAACTG-3' (SEQ ID NO:18), positions 1361-1380; reverse 5'- GCCGCGCGCTAGTATTCAG-3' (SEQ ID NO:19), positions 9420-9438). The resulting 8078 nucleotide RT-PCR product was cloned and sequenced.

[0115] The extreme 3' end of the genome of P129A was determined by ligating the 3' and 5' ends of the viral RNA together and using RT-PCR to amplify the junction fragment. The resulting junction fragments were cloned and sequenced. Briefly, RNA extracted from pelleted virions was treated with tobacco acid pyrophosphatase (to remove 5' cap structures), then self-ligated with T4 RNA ligase (both from Epicentre Technologies). The primers used were 5'-CGCGT-CACAGCATCACCTCAG-3' (SEQ ID NO:20) (forward, positions 15,218-15,239) and either 5'-CGGTAGGTTGGT-TAACACATGAGTT-3' (SEQ ID NO:21) (reverse, positions 656-680) or 5'-TGGCTCTTCGGGCCTATAAAATA-3' (SEQ ID NO:22) (reverse, positions 337-359). All of the resulting clones were truncated at the 5' end of the genome (the most complete came to within 57 nucleotides of the actual 5' end, as revealed by 5' RACE), however two of these clones contained the complete 3' end of the genome including the polyadenosine tail (42 and 55 adenosine residues in length). This completed the sequencing of the cDNA 15,450 base genome of PRRS isolate P129A, including polyA tail, as shown in SEQ ID NO:1.

[01166] Creation of an infectious full-length cDNA clone of P129A: A full-length infectious cDNA clone of P129A, designated pT7P129A, was assembled from four overlapping cloned RT-PCR products. The four RT-PCR products were first T/A cloned into plasmid pCR2.1 (Invitrogen) and transfected into *Escherichia coli* strain DH5-alpha. Bacterial colonies were screened, and those which contained inserts of the expected sizes in the "T7 to M13" orientation were chosen for sequencing and further manipulation. All four cloned RT-PCR products contained one or more non-silent mutations (deviations from the consensus nucleotide sequence for P129A of SEQ ID NO:1 which would result in a change in amino acid sequence of the encoded ORFs). These non-silent mutations (save one at position 12,622 in ORF 2) were repaired by subcloning fragments from other cloned RT-PCR products. The four repaired subgenomic clones were assembled into a full-length clone in a stepwise manner, using available restriction sites (see Figure 1). The 5' and 3' ends of the cDNA corresponding to the P129A genome in pT7P129A were modified by the addition of a T7 promoter and appropriate restriction endonuclease sites. The construction of pT7P129A is described in further detail in the following paragraphs:

[0117] The 5' end of the genome (positions 1-1756), generated by 5'-RACE and cloned into pCR2.1 as described above, was modified to include a T7 promoter immediately upstream of the cDNA corresponding to the P129A genome and a *PacI* site for future cloning. A 3-way ligation was performed using the 1216 bp *DsaI* - *BseRI* fragment of this plasmid (containing bases 27-1242 of P129A), the 4407 bp *BseRI* - *XbaI* fragment of the same plasmid (containing bases 1243-1756 of P129A and the entire plasmid vector up to the *XbaI* site), and the following synthetic double-stranded adapter (SEQ ID NOS:23 and 24):



[0118] The predicted transcript from the T7 promoter includes a single "G" residue from the promoter immediately upstream of the first "A" of the viral genome. A non-silent mutation at position 1230 (A to G) was repaired by replacing the 906 bp *AatII* - *SacI* fragment (bases 740-1645) with the same fragment from another clone. This plasmid was designated "pT7R3A-2".

[0119] The 8078 nucleotide PCR product described above was used to cover bases 1361-9438 of the P129A genome. A 58 bp deletion (positions 2535-2592) and 7 non-silent point mutations were corrected by subcloning fragments from other cloned RT-PCR products, yielding plasmid "pGAP10B-4". The 7837 bp *Bsu*361 - *Spe*I fragment from this plasmid was ligated to the 5482 bp *Bsu*361 - *Spe*I fragment from pT7R3A-2. The resulting plasmid "pT7RG" contains the first

9438 bases of the P129A genome behind the T7 promoter.

[0120] The 2850 nucleotide fragment of ORF1b described above (genome positions 9201-12,050) was corrected to repair non-silent mutations and designated "p1B3A-2". The 2682 bp *Nde*I - *Spe*I fragment of this plasmid was ligated to the 13,249 bp *Nde*I - *Spe*I fragment of pT7RG to yield plasmid "pT71A1B", which contains the first 12,050 bases of the P129A genome.

[0121] The fourth and final fragment of the P129A genome was derived by RT-PCR of ORFs 2 through 7, including the 3' non-translated region and a portion of the polyA tail. The forward primer was 5'-ACTCAGTCTAAGTGCTGGAAAGT-TATG-3' (SEQ ID NO:25) (positions 11,504-11,530) and the reverse primer was 5'-GGGATTAAATATGCATTTTTTTTTTTTTTTTTTTTAAATTGCGGCCGCATGGTTCTCG-3' (SEQ ID NO:26). The reverse primer contains the last 22 bases of the P129A genome (positions 15,374-15,395), a polyA tail of 21 bases, an *Nsi*I site (ATGCAT) and a *Swa*I site (ATTTAAAT). Non-silent point mutations and a single base deletion were repaired by subcloning fragments from other clones. An additional non-silent point mutation at position 12,622 (A to G) was inadvertently introduced at this stage. This results in a change from glutamine to arginine near the C-terminus of the ORF2 protein (amino acid residue 189 of the 256 amino acids in ORF2, which does not affect the overlapping ORF 3). This mutation had no apparent influence on viral growth, in cell culture or in pigs, and was not repaired. This mutation served as a genetic marker to distinguish virus derived from the cDNA clone from possible contamination with parental P129A or other PRRS viruses. The plasmid was designated "p2_7D-4". The structural genes of P129A were added to the rest of the genome by ligating the 3678 bp *Eco*47III - *Spe*I fragment of p2_7D-4 to the 15,635 bp *Eco*47III - *Spe*I fragment of pT71A1B.

[0122] This yields the final construct "pT7P129A", which comprises cDNA corresponding almost identically to the entire genome of P129A (however, with only a 21 base polyA tail, as opposed to 55 base polyA tail) behind a T7 promoter, cloned into the pCR2.1 vector between unique restriction enzyme sites (*Pac*I and *Swa*I). The total length of pT7P129A is 19,313 bp, and it is stable in *E. coli* strain DH5-alpha. pT7P129A contains an A to G non-silent point mutation at position 12,622 that results in an arginine at position 189 of ORF2 rather than a glutamine (as is encoded by SEQ ID NO:1) and a silent C to T mutation at position 1559. Neither of these mutations affected viral growth under the conditions examined, both in cell culture and in pigs. For example, pT7P129A was used for *in vitro* transcription and the resulting RNA transcripts produced live North American PRRS virus when transfected into MARC-145 cells, thus demonstrating that this full-length clone is infectious.

[0123] *In vitro* transcription and transfection of RNA transcripts: In plasmid pT7P129A there are two T7 promoters in tandem upstream of the viral genome. One of these is positioned immediately upstream of the viral genome and was built into the PCR primer as described above. The other is present in the pCR2.1 cloning vector and is located outside of the multiple cloning site (initiating transcription 44 bases upstream of the viral genome). *Pac*I was used to cut between these T7 promoters prior to *in vitro* transcription to generate a transcript that is closer to authentic viral RNA (a single extra G immediately upstream the viral genome, as opposed to 44 extra bases from the distal T7 promoter). In addition, pT7P129A was cut with *Swa*I prior to *in vitro* transcription. The resulting run-off transcripts include a 21 base long polyA tail and nine non-PRRS nucleotides, including an *Nsi*I site (which was not used to linearize the plasmid, since the site also occurs once in the viral genome). The digested plasmid was purified by phenol extraction and ethanol precipitation prior to use.

[0124] A commercial kit (T7 Cap-Scribe, Boehringer Mannheim) was used for *in vitro* transcription. The DNA pellet from above, containing about 0.6 µg of *Pac*I/*Swa*I digested pT7P129A, was resuspended in 20 µl of T7 Cap-Scribe buffer/T7 polymerase and incubated at 37°C for 30 minutes. A portion of the reaction was analyzed by agarose gel electrophoresis and shown to contain full-length RNA transcripts in addition to the expected DNA bands of 15,445 bp and 3868 bp. The *in vitro* transcription reaction was used fresh, immediately following incubation, without purification. Freshly confluent monolayers of MARC-145 cells were washed once in OptiMEM (without serum), and covered with 1 ml per 35mm well of OptiMEM (without serum) containing 500 µg/ml DEAE dextran (molecular weight approx. 500,000, Pharmacia Biotech). *In vitro* transcription reaction (15 µl) was added immediately. After 1 hour at 37°C, the transfection mixture was removed, monolayers were washed once with PBS and overlaid with 1.25% SeaPlaque agarose (FMC corporation) in OptiMEM with 2% fetal bovine serum and antibiotics. After 5 days at 37°C, a single plaque was visible. This virus was designated "rP129A-1" and was expanded on MARC-145 cells and characterized in cell culture and in pigs. Subsequent transfections of *in vitro* transcribed RNA from pT7P129A, using both DEAE dextran and electroporation, have yielded many additional plaques.

[0125] Characterization of recombinant virus rP129A-1: There are no apparent differences in growth kinetics, yield, or plaque morphology between cDNA-derived recombinant virus rP129A-1 and its non-recombinant parent P129A. As discussed above, there are two differences in nucleotide sequence between the coding sequence of pT7P129A and the consensus sequence of P129A (shown in SEQ ID NO:1). Firstly, at position 1559 pT7P129A contains a T, whereas P129A contains a C (this is a silent mutation). Secondly, at position 12,622 pT7P129A contains a G, whereas P129A contains an A (this is the glutamine to arginine change in ORF2 described above). In order to rule out the possibility that rP129A-1 is actually a non-recombinant PRRS virus contaminant, RT-PCR and sequencing were performed on the

regions surrounding these two differences. In the case of both genetic markers, rP129A-1 was identical to plasmid pT7P129A and different from parental virus P129A, thus confirming that rP129A-1 is derived from the infectious cDNA clone.

[0126] Characterization of recombinant virus rP129A-1 in pigs: The cDNA-derived virus rP129A-1 was compared to its non-recombinant parent P129A for its ability to infect and cause clinical disease in young pigs. Three groups of 10 pigs each from a PRRS-negative herd were infected at 4 weeks of age with either P129A, rP129A-1, or mock-infected with cell culture medium. Clinical signs, rectal temperatures, and body weights were monitored. Blood was collected on days 0, 2, 6, 10, and 13 post-infection for determination of serum viremia (by plaque assay on MARC-145 cells, Figure 2) and serum antibody (by ELISA using HerdChek PRRS from IDEXX, Figure 3). Gross and microscopic lesions of the lung were observed upon necropsy. There were no significant differences between the two virus-infected groups, indicating that rP129A-1 replicates in pigs and causes clinical disease which is quantitatively and qualitatively similar to its non-recombinant parent virus.

Example II. Deletion of ORF7 (Nucleocapsid Gene) from the North American PRRS Virus; Preparation of a Negatively-Marked, Replication-Defective Vaccine Thereby.

[0127] The viral nucleocapsid gene (ORF7) was partially deleted from an infectious cDNA clone of the PRRS virus of the present invention. The resulting recombinant modified PRRS virus would be expected to be replication-defective in pigs. This recombinant modified PRRS virus could be used as a vaccine to induce an immune response to the other PRRS virus proteins without the risks of clinical disease, spread to non-vaccinated animals, or reversion to virulence associated with attenuated live vaccines. In addition to being very safe, such a vaccine virus would also be "negatively marked", in the sense that it would allow exposure to field isolates of PRRS virus to be determined serologically, even in the presence of antibody to the vaccine virus. Antibodies to the ORF7 protein are commonly found in the sera of PRRS virus-infected pigs, whereas pigs vaccinated with an ORF7-deleted PRRSV would lack antibodies to the ORF7 protein.

[0128] Deletion of ORF7 from an infectious clone was accomplished as follows: Plasmid p2_7D-4 (see Figure 1) was used as template in PCR to amplify the 5' and 3' flanking regions upstream and downstream of ORF7. The upstream flank forward primer 5'-ATTAGATCTTGCCACCATGGTGGGGAAATGCTTGAC-3' (SEQ ID NO:27) (which binds to genome positions 13,776-13,791 near the beginning of ORF5 and contains additional restriction sites which are irrelevant to the current cloning) and the upstream flank reverse primer 5'-CTTTACGCGTTTGCTTAAGTTATTTGGCGTATTTGACAAGGTTTAC-3' (SEQ ID NO:28) (which binds to genome positions 14,857-14,902 at the junction of ORFs 6 and 7) amplified a fragment of 1147 bp. The reverse primer introduced *MluI* and *AflI* sites and a single base change at position 14,874, destroying the ATG start codon for ORF7 without altering the tyrosine encoded in the overlapping ORF6. For the downstream flank, the forward primer 5'-CAACACGCGTCAGCAAAAGAAAAAGAGGGG-3' (SEQ ID NO:29) (positions 14,884-14,914 near the 5' end of ORF7, introduced an *MluI* site) and reverse primer 5'-GCGCGTTGGCCGATTCATTA-3' (SEQ ID NO:30) (downstream of the viral genome in the pCR2.1 plasmid) amplified a 462 bp fragment. A 3-way ligation was performed, using the 611 bp *BstEII-MluI* fragment of the upstream flank PCR product, the 575 bp *MluI-SpeI* fragment of the downstream flank PCR product, and the 6653 bp *BstEII-SpeI* fragment from plasmid p2_7D-4 (all fragments were gel purified following digestion). The resulting plasmid p2_7Ddelta7+7023 was deleted in the first seven amino acids of ORF7, and lacks a functional ATG start codon. Two new restriction sites which are absent in both the viral genome and the plasmid backbone, *AflI* and *MluI*, have been inserted to facilitate directional cloning of foreign genes into the space previously occupied by the 5' end of ORF7.

[0129] The changes made in p2_7Ddelta7+7023 were incorporated into a full-length genomic clone by ligating the 3683 bp *Eco47III-SpeI* fragment of p2_7Ddelta7+7023 with the 15,214 bp *Eco47III-SpeI* fragment of pCMV-S-p129. The resulting plasmid pCMV-S-p129delta7+7023 was used to transfect cells.

[0130] Since nucleocapsid is essential for viral growth, it is necessary to provide this protein in order to allow generation and replication of an ORF7-deficient PRRS virus. This can be accomplished using a helper virus or a complementing cell line, for example. ORF7-expressing MARC-145 cell lines could be created by stably transfecting cells with a plasmid containing both the ORF7 gene from P129A and the neomycin resistance gene. After selecting for neomycin resistance using the antibiotic G418, single-cell colonies could then be expanded and characterized. Clonal MARC-145-derived cell lines that are positive for ORF7 expression by both immunofluorescence and RT-PCR could be transfected with RNA from pT7P129delta7 in order to generate ORF7-deficient P129 virus.

[0131] Similar strategies can be used to generate PRRS viruses deficient in other structural genes (ORFs 2, 3, 4, 5, or 6), or deficient in all or portions of non-structural genes 1a and 1b. In addition, multiple deletions can be engineered into a single PRRS virus, and these can be grown in complementing cells which provide all necessary functions. Such gene-deficient PRRS viruses are likely to be either partially or completely attenuated in pigs, making them useful as vaccines against PRRS. They can also be used to distinguish vaccinated animals from animals infected with a wild-type PRRS virus as discussed above and/or as vectors for vaccinating animals with epitopes of other porcine pathogens (see

Example III, below).

Example III. Insertion of Heterologous Genes into the North American PRRS Virus Genome; use of PRRS Virus as a Vector, and a Positively-Marked North American PRRS Virus.

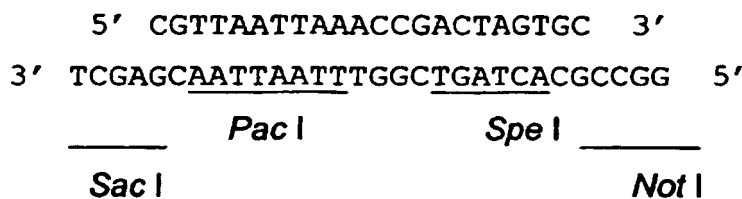
[0132] In Example II, above, *Afl*II and *Mlu*I restriction enzyme sites were inserted into the region formerly occupied by the 5' end of ORF7. These sites are absent in the P129A genome and in the pCR2.1 and pCMV plasmids, and can be used in the directional cloning of foreign (heterologous) genes into the viral genome for expression. Potential leader-junction sites for transcription of the ORF7 subgenomic RNA at positions 14,744-14,749 (ATAACC) and 14,858-14,863 (TAAACC) are not affected by deletion of the ORF7 coding sequence, and can function in transcription of a foreign gene. Foreign (heterologous) genes can include genes from other PRRS virus isolates or genotypes, and/or genes from other non-PRRS pathogens, either pathogens that infect swine or pathogens that infect mammals other than swine, or avians.

[0133] In addition, these foreign genes (or portions thereof) can provide antigenic epitopes which are not normally found in swine. Such epitopes can be used to "positively mark" a vaccine, so that successful vaccination can be monitored serologically, even in the presence of antibody to field or conventional vaccine strains of PRRS virus. A positive marker needs not be a separate expression cassette. An antigenic epitope can be fused to a structural gene of the PRRS virus. For example, the upstream flank reverse primer described in Example I, above, can be extended in such a way as to add a carboxyl-terminal fusion of a non-PRRS virus antigenic epitope to the ORF6 membrane protein. The presence of antibody to this epitope in swine indicates successful vaccination.

Example IV. Cellular Expression of a PRRS Virus by Direct Transfection of cDNA into Cells.

[0134] The eukaryotic expression vector pCMV-MC1 (SEQ ID NO:31) was derived from the commercially available plasmid pCMVbeta (Clontech) by replacing the LacZ coding sequence between two *Not* I sites with a linker containing *Not* I, *Eco*R V, *Avr* II, *Bgl* II, *Cla* I, *Kpn* I, *Pac* I, *Nhe* I, *Swa* I, *Sma* I, *Spe* I and *Not* I sites. Modification of the human CMV immediate early promoter was accomplished by substituting the sequence between the *Sac* I and the second *Not* I sites of pCMV-MC1 with a synthetic linker (shown below). The linker contains a half site for *Sac* I following by *Pac* I, *Spe* I and a half site for *Not* I. After annealing the two single stranded oligonucleotides, the linker was cloned into pCMV-MC1 between the *Sac* I and *Not* I sites, and a selected clone was designated pCMV-S1. The *Spe* I site of pCMV-S1 could not be cut, possibly due to a mistake in the oligo sequence. Therefore, the fragment between *Pac* I and *Hind* III in pCMV-S1 was replaced with *Pac* I (at position 877) - *Hind* III (at position 1162) fragment from pCMV-MC1. Thus, a *Spe* I site was regained. This final construct (pCMV-S) was used to clone the full length P129 genome.

[0135] Linker sequence (SEQ ID NOS:32 and 33):



[0136] The sequence immediately upstream of the 5' end of the P129 genome was modified to contain proper spacing and a convenient restriction enzyme site (*Pac* I). This was done by designing appropriate PCR primers (SEQ ID NOS: 34 and 35) for amplification from pT7P129. After digestion with *Pac* I and *Aat* II, this PCR fragment was subcloned into the *Pac* I and *Aat* II sites of pT7RG (Fig. 1). The resulting plasmid was designated pT7RG-deltaT7.

[0137] The final construction was completed by subcloning the viral sequences from pT7RG-deltaT7 at the *Pac* I and *Nde* I sites into pT7P129, creating pT7P129-deltaT7. The full length P129 genome was digested from pT7P129-deltaT7 at *Pac* I and *Spe* I and transferred into pCMV-S at the *Pac* I and *Spe* I sites. This constructed was named pCMV-S-P129.

[0138] The sequence of the region of modification between the CMV promoter TATA box and the 5' end of the P129 sequence in pCMV-S-P129 is shown in SEQ ID NO:36 and schematically presented below:

5' TATATAAGCAGAGCTCGTTAATTAAACCGTCATGACGTATAGGTGTTGGC 3'

TATA box Sac I Pac I Start of P129

[0139] To test the use of the CMV promoter to initiate PRRS virus infection in cells, pCMV-S-P129 plasmid DNA (0.5 or 1.0 µg) was transfected into MARC-145 cells by lipofection using Lipofectamine™ (Life Technologies Inc.). PRRS virus specific cytopathic effect was observed after transfection and the presence of PRRS virus antigen was determined by the immunofluorescent antibody test.

[0140] PRRS virus was generated efficiently from pCMV-S-P129, and the progeny virus could be passaged on MARC-145 cells. This demonstrates that a PRRS virus infection can be initiated directly from a plasmid cDNA encoding a PRRS virus, without an *in vitro* transcription step. Furthermore, pCMV-S-P129 generated a greater amount of progeny virus compared to plasmids wherein the 3' end of the pCMV promoter was not immediately in front of the start of the sequence encoding the North American PRRS virus.

Example V. Deletion of ORF4 from the North American PRRS Virus; Preparation of a Replication-Defective Vaccine Thereby.

[0141] A portion of the gene for ORF4, which encodes a membrane glycoprotein, was deleted from an infectious cDNA clone of the PRRS virus of the present invention. The resulting recombinant modified PRRS virus is expected to be replication-defective in pigs and to induce an immune response to the other PRRS virus proteins without the risks of clinical disease, spread to non-vaccinated animals, or reversion to virulence associated with attenuated live vaccines.

[0142] Deletion of ORF4 from an infectious clone was accomplished as follows. Plasmid p2_7D-4 (see Figure 1) was used as template in PCR to amplify the 5' and 3' flanking regions upstream and downstream of ORF4. The upstream flank forward primer was 5'-AGGTCGACGGCGGCAATTGGTTTCACCTAGAGTGGCTGCGTCCCTTCT-3' (SEQ ID NO:37). This primer binds to genome positions 13194-13241, near the beginning of ORF4, and introduces a mutation at position 13225 which destroys the ATG start codon of ORF4 without altering the overlapping amino acid sequence of ORF3. The upstream flank reverse primer was 5'-TCTTAAGCATTGGCTGTGATGGTGATATAC-3' (SEQ ID NO:38). This primer binds to genome positions 13455-13477 within the ORF4 coding region, downstream of ORF3, and introduces an *Afl*III site. For the downstream flanking region, the forward primer was 5'-CTTCTTAAGTCCACGCGTTTCTTCTTGCCTTTCTATGCTTCT-3' (SEQ ID NO:39). This primer binds to genome positions 13520-13545 in the middle of ORF4, and introduces *Afl*III and *Mlu*I sites for directional cloning of foreign genes. The reverse primer was 5'-TGCCCGGTCCCTTGCCTCT3' (SEQ ID NO:40). This primer binds to genome positions 14981-14999 in the ORF7 coding sequence. A three-way ligation was performed using the *Sal*I-*Afl*III fragment of the upstream flank PCR product, the *Afl*III-*Bst*EII fragment of the downstream flank PCR product, and the *Sal*I-*Bst*EII fragment from plasmid p2_7D-4. All fragments were gel-purified following digestion. The resulting plasmid p2_7D-4delta4N has 42 bases of the central portion of ORF4 deleted and replaced with a 15 base artificial cloning site. The cloning site contains two restriction sites (*Afl*III and *Mlu*I) that are absent from both the viral genome and the plasmid backbone. These can be used to facilitate directional cloning of foreign genes into the space previously occupied by ORF4. The cloning site also contains a stop codon (TAA) that is in frame with ORF4 and further assures that functional ORF4 protein is not produced.

[0143] It was found that a more extensive deletion of the ORF4 coding sequence could be made without interfering with expression of the downstream ORF5 envelope gene. In this case a shorter downstream flanking region was amplified by PCR using the same template and reverse primer, and using forward primer 5'-GTTTACGCGTCGCTCCTTGGTGGTTCG-3' (SEQ ID NO:41). This primer binds to genome positions 13654-13669 near the 3' end of ORF4, and contains an *Afl*III site. Two-way ligation between the *Afl*III-*Bst*EII fragment of the downstream flank PCR product and the *Afl*III-*Bst*EII fragment from plasmid p2_7D-4delta4N yielded the new plasmid p2_7D-4delta4NS. This plasmid has 176 bases of the ORF4 coding sequence deleted and replaced with the 15 base cloning site.

[0144] The changes made in p2_7D-4delta4N and p2_7Ddelta4NS were incorporated into the full-length genomic clone by replacing the *Bsr*GI-SpeI fragment from pCMV-S-P129 with the modified *Bsr*GI-SpeI fragments from p2_7D-4delta4N and p2_7D-4delta4NS. The resulting plasmids pCMV-S-P129delta4N and pCMV-S-P129delta4NS were used to transfect cells.

[0145] In contrast to pCMV-S-P129, transfection of MARC-145 cells with plasmids pCMV-S-P129delta4N or pCMV-S-P129delta4NS did not result in viral plaques or fluorescent foci. Individual transfected cells could be seen to be producing the ORF7 nucleocapsid protein, suggesting that the ORF4 gene product is not required for RNA replication or expression of viral genes, but is essential for release of infectious progeny virus. Since deletion of ORF4 is lethal to virus replication, it is necessary to provide this protein. This can be accomplished by using a complementing cell line. We created ORF4-expressing MARC-145 cell lines by stable transfecting cells with a plasmid containing both ORF4

and the neomycin resistance gene. After selection for neomycin resistance using the antibiotic G418, single-cell colonies were expanded and characterized. After transfection with pCMV-S-P129delta4NS, three ORF4-expressing cell clones yielded live virus that could be propagated in these cells but not in MARC-145 cells. One of these, MARC400E9, was further characterized. Immunofluorescent staining for viral nucleocapsid in MARC400E9 cells transfected with plasmid pCMV-S-P129delta4NS was positive.

Example VI. Use of Gene-Deleted PRRS Virus as a Vector for Expression of Foreign Genes.

[0146] In order to determine whether heterologous genes can be expressed from a gene-deleted PRRS virus, we inserted a copy of green fluorescent protein (GFP) into the site of ORF4 deletion in plasmid pCMV-S-P129delta4N. The GFP gene was amplified from a commercially available plasmid vector using PCR primers that introduced an *Afl* site at the 5' end of the gene and an *Mlu* site at the 3' end of the gene. The resulting plasmid pCMV-S-P129delta4N-GFP was used to transfect MARC-145 and MARC400E9 cells. As anticipated, MARC-145 cells did not support replication of the ORF4-deleted virus. Single green cells resulting from primary transfection were seen under the UV scope, indication that GFP was being expressed, but the virus did not spread to neighboring cells and no CPE was observed. In contrast, large green foci were observed in transfected MARC400E9 cells. Visible plaques formed and merged, destroying the monolayer. These results indicate that foreign genes can be expressed from ORF4-deleted PRRS virus, and that increasing the size of the viral genome by 692 bases (4.5%) does not interfere with packaging of the viral RNA into infectious particles.

Example VII. Use of Replication-Competent PRRS Virus as a Vector for Expression of Foreign Genes.

[0147] In order to determine whether heterologous genes can be expressed from a replication-competent PRRS virus, we inserted a copy of GFP into the region between ORF1b and ORF2. Since the leader/junction (UJ) sequence for ORF2 lies within ORF1b, this UJ sequence was used to drive expression of the GFP gene and a copy of the ORF6 UJ sequence was inserted downstream from GFP to drive expression of ORF2.

[0148] Plasmid p2_7D-4 (see Figure 1) was used as template in PCR to amplify the 5' and 3' flanking regions upstream and downstream of the insertion site. The upstream flank forward primer was 5'-AACAGAAGAGTTGTCGGGTCCAC-3' (SEQ ID NO:42). This primer binds to genome positions 11699-11721 in ORF1b. The upstream flank reverse primer was 5'-GCTTTGACGCGTCCCCACTTAAGTTCAATTCAGGCCTAAAGTTGGTTCA-3' (SEQ ID NO:43). This primer binds to genome positions 12031-12055 in ORF1b and adds *Afl* and *Mlu* sites for directional cloning of foreign genes between ORF1b and ORF2. The downstream flank forward primer was 5'-GCGACGCGTGTTCCGTGGCAACCCCTTAACCAGAGTTTCAGCGGAACAATGAAAT-GGGGTCTATACAAAGCCTCTTCGACA-3' (SEQ ID NO: 44). This primer binds to genome positions 12056-12089 in ORF2 and contains an *Mlu* site followed by the 40 bases that precede the start of ORF6 (containing the ORF6 L/J sequence). The downstream flank reverse primer was 5'- AACAGAACGGCACGATACACCACAAA- 3' (SEQ ID NO: 45). This primer binds to genome positions 13819-13844 in ORF5. A three- way ligation was performed using the *Eco*47III- *Mlu* fragment of the upstream flank PCR product, the *Mlu*- *Bsr*GI fragment from the downstream flank PCR product, and the *Eco*47III- *Bsr*GI fragment from pCMV- S- P129. The resulting plasmid pCMV- S- P129-1bMCS2 contains the entire P129 genome with a cloning site and an additional UJ site between ORF1b and ORF2. The plasmid produces functionally normal virus when transfected into MARC- 145 cells.

[0149] The GFP gene from a commercially available plasmid was PCR amplified using primers that add an *Afl* site to the 5' end and an *Mlu* site to the 3' end of the gene. After digestion of the PCR fragment and pCMV-SP129-1bMCS2 with *Afl* and *Mlu*, the insert was ligated into the vector to yield plasmid pCMV-S-P129-1bGFP2. This plasmid produced green plaques when transfected into MARC-145 cells. The resulting virus could be passaged onto MARC-145 cells and continued to produce green plaques when observed under the UV scope. Thus, foreign genes may be expressed from replication-competent PRRS virus vectors. The P129-1bGFP2 virus contains a genome which is 774 bases (5%) longer than that of its P129 parent, yet it is packaged normally.

DEPOSIT OF BIOLOGICAL MATERIALS

[0150] The following biological material was deposited with the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia, 20110-2209, USA, on November 19, 1998 and were assigned the following accession numbers:

Plasmid	Accession No.
plasmid pT7P129A	203488

Table continued

Plasmid	Accession No.
plasmid pCMV-S-P129	203489

5

SEQUENCE LISTING

[0151]

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<210> 3

<211> 4392

<212> DNA

<213> Artificial Sequence

<220>

5 <223> Description of Artificial Sequence: cDNA of Open Reading Frame 1b of North American PRRS Virus Genome.

<400> 3

10

15

20

25

30

35

40

45

50

55

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 cctgtgaatt taaaagtggc cagtgaaggt gagctgaaag acgcggtcga gcacaaccaa 180
 5 caccgcggtt caagaccggg tgacggtggg gttgtgctcc tgcgttcgcg agttccttcg 240
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 ccggggaaca ctgggatcga tggcacgctt tgggactttg aggccgaggc caccaaagag 360
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ggcctgaatt ga 4392

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<210> 4

<211> 771

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: cDNA of Open Reading Frame 2 of North American PRRS Virus Genome.

<400> 4

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 5 tcaccatcgc cggttggctg gtggtctttt gcatcagatt ggtttgctcc gcggtattcc 180
 gtgcgcgccc tgccattcac cctgagcaat tacagaagat cctatgaggc ctttctttct 240
 cagtgccggg tggacattcc cacctggggg gtaaaacacc ctttggggat gttttggcac 300
 cataaggtgt caaccctgat tgatgaaatg gtgtcgcgtc gaatgtaccg catcatggaa 360

aaagcagggc aagctgcctg gaaacaggtg gtgagcgagg ctacgctgtc tcgcattagt 420
 agtttgatg tgggtggctca ttttcaacat cttgcgcga ttgaagccga gacctgtaa 480
 15 tatttgctt ctcgactgcc catgctacac aacctgcga tgacagggc aaatgtaacc 540
 atagtgtata atagcacttt aaatcaggtg tttgctatct ttccaacccc tggttcccg 600
 ccaaagcttc atgattttca gcaatggcta atagctgtac attcctccat attttcctct 660
 gttgcagctt cttgtactct ttttgttggt ctgtgggtgc gggttccaat gctacgtact 720
 20 gtttttggtt tccgctgggt aggggcaatt tttcttcga actcatggtg a 771

<210> 5

<211> 765

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: cDNA of Open Reading Frame 3 of North American PRRS Virus Genome.

<400> 5

atggctaata gctgtacatt cctccatatt ttctctgtt gcagcttctt gtactctttt 60
 tgttgtgctg tgggtgcggg ttccaatgct acgtactgtt tttggtttcc gctggttagg 120
 40 ggcaattttt ctttcgaact catggtgaat tacacggtgt gtccaccttg cctcaccgga 180
 caagcagccg ctgaggtcct tgaaccggg aggtctcttt ggtgcaggat agggcatgac 240
 cgatgtgggg aggacgatca cgacgaacta gggttcatgg ttccgcctgg cctctccagc 300
 gaaagccact tgaccagtgt ttacgcctgg ttggcgttcc tgccttcag ctacacggcc 360
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 caccaattca tctgcgccgt tcatgacggg cagaacacca ccttgcctcg ccatgacaat 480
 atttcagccg tatttcagac ctactatcaa catcaggtcg acggcgga tttggtttcac 540
 ctagaatggc tgcgtccctt cttttcctct tggttggtt taaatgtttc gtggtttctc 600
 50 aggcgttcgc ctgcaagcca tgtttcagtt cgagtcttcc agacatcaaa accaacta 660
 ccgcagcatc aggttttggt gtcctccagg acatcagctg ccttaggcgt ggcgactcgt 720
 ctttccgac gatccgcaa agctctcaat gccgcacggc gatag 765

<210> 6

<211> 537

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: cDNA of Open Reading Frame 4 of North American PRRS Virus Genome.

<400> 6

```

atggctgcgt cccttctttt cctcttggtt ggttttaaat gtttcgtggt ttctcaggcg 60
ttcgccgtgca agccatgttt cagttcgagt ctttcagaca tcaaaaccaa cactaccgca 120
gcatcagggt ttgttgctct ccaggacatc agctgcctta ggcatggcga ctgcctcttt 180
ccgacgattc gcaaaagctc tcaatgccgc acggcgatag ggacacccgt gtatatcacc 240

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atcacagcca atgtgacaga tgagaattac ttacattctt ctgatctcct catgctttct 300
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tttaccacaac gtccttggtt ggtcgatcat gtgcggctgc ttcatttcat gacacctgag 480
accatgaggt gggcaaccgt tttagcctgt ctttttgcca tcctactggc aatttga 537

```

<210> 7

<211> 603

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: cDNA of Open Reading Frame 5 of North American PRRS Virus Genome.

<400> 7

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atgttgggga aatgcttgac cgcgggctgt tgctcgcgat tgctttcttt gtggtgtatc 60
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ttgatattata acttgacgct atgtgagctg aatggcacag attggctggc agaaaaattt 180
gattgggcag tggagacttt tgtcatcttt cccgtgttga ctacattgt ttcctatggt 240
gcactcacca ccagccattt ccttgacaca gttggtctgg ttactgtgtc caccgccggg 300
ttttatcacg ggcggtatgt cttgagtagc atctacgcgg tctgtgctct ggctgcgttg 360
atttgcttcg ttattaggct tgcaagaac tgcatgtcct ggcgtactc ttgtaccaga 420
tataccaact tccttctgga cactaagggt agactctatc gttggcggtc gcccgttatc 480
atagaaaaag ggggtaaggt tgaggtcgaa ggtcacctga tcgacctcaa aagagttgtg 540
cttgatgggt ccgtggcaac ccctttaacc agagtttcag cggaacaatg gggtcgtctc 600
tag 603

```

<210> 8

<211> 525

<212> DNA

5 <213> Artificial Sequence

<220>

10 <223> Description of Artificial Sequence: cDNA of Open Reading Frame 6 of North American PRRS Virus Genome.

<400> 8

15 atgggggtcgt ctctagacga cttttgccat gatagcacgg ctccacaaaa ggtgcttttg 60
gcgtttttcca ttacctacac gccagtaatg atatatgctc taaaggtaag tcgcggccga 120
ctactagggc ttctgcacct tttgatcttt ctgaattgtg cttttacctt cgggtacatg 180
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20 ttgtgcttgc taggccgcaa gtacattctg gcccctgccc accacgtcga aagtgccgcg 360
ggccttcatc cgattgcggc aaatgataac cacgcatttg tcgtccggcg tcccggtcc 420
actacgggta acggcacatt ggtgcccggg ttgaaaagcc tcgtgttggg tggcagaaaa 480

25 gctgttaaac agggagtggg aaaccttgct aaatatgcc aataa 525

<210> 9

30

<211> 372

<212> DNA

35 <213> Artificial Sequence

<220>

40 <223> Description of Artificial Sequence: cDNA of Open Reading Frame 7 of North American PRRS Virus Genome.

<400> 9

45 atgccaaata acaacggcaa gcagcaaaag aaaaagaagg ggaatggcca gccagtcaat 60
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ccctcagcat ga 372

<210> 10

55

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

5 <223> Description of Artificial Sequence: Primer, forward strand, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 10
acagttcggg gatctatg 18

10 <210> 11

<211> 17

15 <212> DNA

<213> Artificial Sequence

<220>

20 <223> Description of Artificial Sequence: Primer, reverse strand, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 11
cagattcaga tgttcaa 17

25 <210> 12

<211> 24

30 <212> DNA

<213> Artificial Sequence

35 <220>

<223> Description of Artificial Sequence: Primer used for determining cDNA corresponding to North American PRRS virus genome.

40 <400> 12
acctcgtgct gtagccgaa tctc 24

<210> 13

45 <211> 24

<212> DNA

<213> Artificial Sequence

50 <220>

<223> Description of Artificial Sequence: Primer, used for determining cDNA corresponding to North American PRRS virus genome.

55 <400> 13
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<210> 14

<211> 24

5 <212> DNA

<213> Artificial Sequence

<220>

10 <223> Description of Artificial Sequence: Primer, forward strand, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 14

15 gatgactggg ctactgacga ggat 24

<210> 15

<211> 21

20 <212> DNA

<213> Artificial Sequence

25 <220>

<223> Description of Artificial Sequence: Primer, reverse strand, used for determining cDNA corresponding to North American PRRS virus genome.

30 <400> 15

agagcggctg ggatgacact g 21

<210> 16

35 <211> 22

<212> DNA

<213> Artificial Sequence

40 <220>

<223> Description of Artificial Sequence: Primer, used for determining cDNA corresponding to North American PRRS virus genome.

45 <400> 16

ccggggaagc cagacgattg aa 22

<210> 17

50 <211> 24

<212> DNA

55 <213> Artificial Sequence

<220>

EP 1 018 557 B9

<223> Description of Artificial Sequence: Primer, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 17

5 agggggagca aagaaggggt catc 24

<210> 18

<211> 20

10

<212> DNA

<213> Artificial Sequence

15

<220>

<223> Description of Artificial Sequence: Primer forward strand, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 18

20 agcacgctct ggtgcaactg 20

<210> 19

25

<211> 19

<212> DNA

<213> Artificial Sequence

30

<220>

<223> Description of Artificial Sequence: Primer, reverse strand, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 19

35 gccgcggcgt agtattcag 19

<210> 20

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<211> 22

<212> DNA

<213> Artificial Sequence

45

<220>

<223> Description of Artificial Sequence: Primer, forward strand, used for determining cDNA corresponding to North American PRRS virus genome.

<400> 20

50 cgcgtcacag catcaccctc ag 22

<210> 21

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<211> 25

<212> DNA

<213> Artificial Sequence

5 <220>

<223> Description of Artificial Sequence: Primer, reverse strand, used for determining cDNA corresponding to North American PRRS virus genome.

10 <400> 21
cggtagggtg gttacacat gagtt 25

<210> 22

15 <211> 23

<212> DNA

<213> Artificial Sequence

20 <220>

<223> Description of Artificial Sequence: Primer, reverse strand, used for determining cDNA corresponding to North American PRRS virus genome.

25 <400> 22
tggctcttcg ggcctataaa ata 23

<210> 23

30 <211> 56

<212> DNA

35 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Strand of synthetic doublestranded adapter used in pT7P129A.

40 <400> 23
ctagattaat taatacgact cactataggg atgacgtata ggtgttgct ctatgc 56

<210> 24

45 <211> 56

<212> DNA

50 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Strand of synthetic double-stranded adapter used in pT7P129A.

55 <400> 24
taattaatta tgctgagtga tatccctact gcatatccac aaccgagata cggtgc 56

<210> 25

<211> 27

5 <212> DNA

<213> Artificial Sequence

<220>

10 <223> Description of Artificial Sequence: Primer, forward strand, used in preparing pT7P129A.

<400> 25
actcagtcta agtgctggaa agttatg 27

15 <210> 26

<211> 59

20 <212> DNA

<213> Artificial Sequence

<220>

25 <223> Description of Artificial Sequence: Primer, reverse strand, used in preparing pT7P129A.

<400> 26
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30 <210> 27

<211> 36

35 <212> DNA

<213> Artificial Sequence

<220>

40 <223> Description of Artificial Sequence: Primer, forward strand, used for synthesizing region flanking North American PRRS virus ORF7 upstream.

<400> 27
attagatctt gccacatgg tggggaaatg cttgac 36

45 <210> 28

<211> 46

50 <212> DNA

<213> Artificial Sequence

55 <220>

<223> Description of Artificial Sequence: Primer, reverse strand, used for synthesizing region flanking North American PRRS virus ORF7 upstream.

<400> 28
 ctttacgcgt ttgcttaagt tatttggcgt atttgacaag gtttac 46

5

<210> 29
 <211> 30
 <212> DNA

10

<213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Primer, forward strand, used for synthesizing region flanking North American PRRS virus ORF7 downstream.

15

<400> 29
 caacacgcgt cagcaaaaga aaaagaaggg g 31

20

<210> 30
 <211> 20
 <212> DNA

25

<213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Primer, reverse strand, used for synthesizing region flanking North American PRRS virus ORF7 downstream.

30

<400> 30
 gcgcgttggc cgattcatta 20

35

<210> 31
 <211> 37
 <212> DNA

40

<213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: Upper primer used in preparing pCMV-S-P129.

45

<400> 31
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50

<210> 32
 <211> 3796
 <212> DNA

55

<213> Plasmid

<220>

<223> Description of Plasmid: pCMV-MCI

5 <400> 32

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35

40

45

50

55

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55 <210> 33

<211> 22

<212> DNA

<213> Artificial Sequence

5 <220>

<223> Description of Artificial Sequence: Strand from synthetic linker used in the preparation of pCMV-S-P129,

10 <400> 33
cgtaataa accgactagt gc 22

<210> 34

15 <211> 30

<212> DNA

<213> Artificial Sequence

20 <220>

<223> Description of Artificial Sequence: Strand from synthetic linker used in the preparation of pCMV-S-P129.

25 <400> 34
tcgagcaatt aattggctg atcacgccg 30

<210> 35

30 <211> 24

<212> DNA

<213> Artificial Sequence

35 <220>

<223> Description of Artificial Sequence: Lower primer used in preparing pCMV-S-P129.

40 <400> 35
cggggacggt tcaaatttc actt 24

<210> 36

45 <211> 50

<212> DNA

<213> Artificial Sequence

50 <220>

<223> Description of Artificial Sequence: Portion of plasmid pCMV-S-P129, in 5' to 3' direction, immediately prior to P129 genome.

55 <400> 36
tatataagca gagctcgta attaaaccgt catgacgtat aggtgttggc 50

<210> 37

<211> 48

<212> DNA

5 <213> Artificial Sequence

<220>

10 <223> Description of Artificial Sequence:Primer, forward, used for synthesizing upstream flanking region to ORF4

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15 <210> 38

<211> 30

<212> DNA

20 <213> Artificial Sequence

<220>

25 <223> Description of Artificial Sequence:Primer, reverse, used for synthesizing upstream flanking region to ORF4

<400> 38
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30 <210> 39

<211> 44

<212> DNA

35 <213> Artificial Sequence

<220>

<220>

40 <223> Description of Artificial Sequence:Primer, forward, used for synthesizing downstream flanking region to ORF4

<400> 39
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45 <210> 40

<211> 19

50 <212> DNA

<213> Artificial Sequence

<220>

55 <223> Description of Artificial Sequence:Primer, reverse, used for synthesizing downstream flanking region to ORF4

<400> 40

tgcccgggtcc cttgcctct 19

<210> 41

5 <211> 26

<212> DNA

10 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer, forward, used for synthesizing downstream flanking region to ORF4

15 <400> 41

gtttacgcgt cgctccttgg tggtcg 26

<210> 42

20 <211> 23

<212> DNA

25 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer, forward, used for synthesizing upstream flanking region to insertion site between ORF1b and ORF2

30

<400> 42

aacagaagag ttgtcgggtc cac 23

<210> 43

35

<211> 49

<212> DNA

40 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer, reverse, used for synthesizing upstream flanking region to insertion site between ORF1b and ORF2

45

<400> 43

gctttgacgc gtccccactt aagtcaatt caggcctaaa gttggtca 49

50 <210> 44

<211> 82

<212> DNA

55

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer, forward, used for synthesizing downstream flanking region to insertion site between ORF1b and ORF2

<400> 44

gcgacgcgtg ttccgtggca acccctttaa ccagagtttc agcggaaaca tgaaatgggg 60
tctatacaaa gcctcttcga ca 82

<210> 45

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Primer, reverse, used for synthesizing downstream flanking region to insertion site between ORF1b and ORF2

<400> 45

aacagaacgg cagatacac cacaaa 26

Claims

1. An isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO:1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine.
2. An isolated infectious RNA molecule encoded by an isolated polynucleotide molecule according to claim 1. which infectious RNA molecule encodes a North American PRRS virus.
3. An isolated polynucleotide molecule according to claim 1 in the form of a plasmid.
4. A transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus, wherein said DNA sequence is SEQ ID NO:1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine, which transfected host cell, is capable of expressing the encoded North American PRRS virus.
5. A plasmid capable of directly transfecting a suitable host cell and expressing a North American PRRS virus from the suitable host cell so transfected, which plasmid comprises a) a DNA sequence encoding an infectious RNA molecule encoding the North American PRRS virus, and wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine and b) a promoter capable of transcribing said infectious RNA molecule in said suitable host cell.
6. A method for generating a North American PRRS virus, which method comprises transfecting a suitable host cell with a plasmid according to claim 5 encoding the North American PRRS virus and obtaining the North American PRRS virus generated by the transfected host cell.

7. An isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that when it infects a porcine animal it is unable to produce PRRS in the animal, wherein said DNA sequence is SEQ ID NO:1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine, except for that it contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS.
8. A transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that when it infects a porcine animal it is unable to produce PRRS in the animal, wherein said DNA sequence is SEQ ID NO:1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine, except for that it contains one or more mutations that genetically disable the encoded PRRS virus in its ability to produce PRRS, which transfected host cell is capable of expressing the encoded genetically modified North American PRRS virus.
9. A vaccine for protecting a porcine animal from infection by a PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule according to claim 7, or said infectious RNA molecule, or said polynucleotide molecule in the form of a plasmid, or a viral vector comprising a nucleotide sequence corresponding to claim 7 encoding a North American PRRS virus that is able to elicit an effective immunoprotective response against infection by a PRRS virus, in an amount effective to produce immunoprotection against infection by a PRRS virus; and a carrier acceptable for veterinary use.
10. An isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more heterologous antigenic epitopes, wherein the DNA sequence encoding the RNA molecule encoding the North American PRRS virus is SEQ ID NO:1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine, except for that it comprises one or more further nucleotide sequences that each encode a heterologous antigenic epitope, and wherein each heterologous antigenic epitope is capable of inducing an effective immunoprotective response against a particular pathogen in a mammal or a bird.
11. An isolated polynucleotide molecule according to claim 10 in the form of a plasmid.
12. A transfected host cell comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more heterologous antigenic epitopes, wherein the DNA sequence encoding the RNA molecule encoding the North American PRRS virus is SEQ ID NO:1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine except for that it comprises one or more further nucleotide sequences that each encode a heterologous antigenic epitope, and wherein each heterologous antigenic epitope is capable of inducing an effective immunoprotective response against a particular pathogen in a mammal or a bird.
13. A vaccine for protecting a mammal or a bird from infection by a pathogen, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule of claim 10, or said infectious RNA molecule, or a plasmid according to claim 11, in an amount effective to produce immunoprotection against infection by the pathogen from which the heterologous antigenic epitope or epitopes thereof or encoded thereby are derived; and a carrier acceptable for pharmaceutical or veterinary use.
14. An isolated polynucleotide molecule according to claim 10 wherein the DNA sequence encoding the infectious RNA molecule encoding the genetically modified North American PRRS virus comprises one or more further mutations that disable the encoded genetically modified North American PRRS virus in its ability to produce PRRS in a swine animal.
15. An isolated polynucleotide molecule according to claim 14 in the form of a plasmid.

16. A vaccine for protecting a porcine animal from infection by a PRRS virus and from infection by a swine pathogen other than a North American PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule according to claim 14, or said infectious RNA molecule, or a plasmid according to claim 15, in an amount effective to produce immunoprotection against infection by a PRRS virus and against infection by the pathogen from which the heterologous antigenic epitope or epitopes thereof or encoded thereby are derived; and a carrier acceptable for veterinary use.
17. An isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified such that it comprises one or more detectable heterologous antigenic epitopes, wherein the DNA sequence encoding the infectious RNA molecule is the same as the sequence SEQ ID NO:1 or the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine except for that it comprises one or more further nucleotide sequences that each encode a detectable heterologous antigenic epitope.
18. A vaccine for protecting a porcine animal from infection by a PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule according to claim 17, comprising one or more detectable heterologous antigenic epitopes, treated or further genetically modified so that it is unable to produce PRRS in a porcine animal yet able to elicit an effective immunoprotective response against a PRRS virus in the porcine animal; or said infectious RNA molecule, wherein the genetically modified North American PRRS virus encoded thereby comprising one or more detectable heterologous antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or an isolated polynucleotide according to claim 17, in the form of a plasmid, wherein the genetically modified North American PRRS virus encoded thereby comprising one or more detectable heterologous antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or a viral vector comprising a nucleotide sequence encoding an infectious RNA molecule encoding such a genetically modified North American PRRS virus, wherein the genetically modified North American PRRS virus encoded thereby comprising one or more detectable heterologous antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; in an amount effective to produce immunoprotection against infection by a PRRS virus; and a carrier acceptable for veterinary use.
19. An isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a North American PRRS virus that is genetically modified so that it lacks a detectable antigenic epitope, wherein said DNA sequence is SEQ ID NO:1, or the sequence beginning with and including nucleotide 1 through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine except for that it lacks one or more DNA sequences encoding a detectable antigenic epitope.
20. A vaccine for protecting a porcine animal from infection by a PRRS virus, which vaccine comprises a genetically modified North American PRRS virus encoded by an infectious RNA molecule encoded by a polynucleotide molecule according to claim 19, lacking one or more detectable antigenic epitopes, treated or further genetically modified so that it is unable to produce PRRS in a porcine animal yet able to elicit an effective immunoprotective response against a PRRS virus in the porcine animal; or said infectious RNA molecule, wherein the genetically modified North American PRRS virus encoded thereby lacking one or more detectable antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or an isolated polynucleotide molecule, in the form of a plasmid, wherein the genetically modified North American PRRS virus encoded thereby lacking one or more detectable antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; or a viral vector comprising a nucleotide sequence encoding an infectious RNA molecule encoding such a genetically modified North American PRRS virus, wherein the genetically modified North American PRRS virus encoded thereby lacking one or more detectable antigenic epitopes comprises further genetic modifications so that it is unable to produce PRRS yet able to elicit an effective immunoprotective response against a PRRS virus; in an amount effective to produce immunoprotection against infection by a PRRS virus; and a carrier acceptable for veterinary use.

21. An isolated polynucleotide molecule comprising one or more nucleotide sequences that each encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA molecule corresponding to SEQ ID NO:1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine.
22. A transfected host cell comprising one or more nucleotide sequences that each encode a peptide encoded by a North American PRRS virus, wherein the genome sequence of said North American PRRS virus is the same as an RNA molecule corresponding to SEQ ID NO:1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine, which transformed cell is capable of expressing said peptide or peptides.
23. A method for preparing a genetically modified North American PRRS virus that is capable of eliciting an immunoprotective response in a mammal or a bird vaccinated therewith, which method comprises obtaining an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a wild-type North American PRRS virus wherein said DNA sequence is SEQ ID NO: 1 or the sequence beginning with and including nucleotide I through and including nucleotide 15,416 of SEQ ID NO:1, except that the nucleotide corresponding to nucleotide 12,622 of SEQ ID NO:1 is a guanine instead of an adenine and the nucleotide corresponding to nucleotide 1,559 of SEQ ID NO:1 is a thymine instead of a cytosine, genetically mutating the DNA sequence encoding the infectious RNA molecule encoding the wild-type North American PRRS virus so as to obtain an isolated polynucleotide molecule comprising a DNA sequence encoding an infectious RNA molecule encoding a genetically modified North American PRRS virus, which virus remains able to elicit an effective immunoprotective response against infection by the wild-type North American PRRS virus in a mammal or a bird, and expressing the genetically modified North American PRRS virus from the isolated polynucleotide molecule so obtained.
24. A vaccine for protecting a mammal or a bird from infection by a North American PRRS virus, which comprises a genetically modified North American PRRS virus according to claim 23 in an amount effective to elicit an effective immunoprotective response against the wild-type North American PRRS virus in a mammal or a bird vaccinated therewith, and a carrier acceptable for pharmaceutical or veterinary use.

Patentansprüche

1. Isoliertes Polynucleotid-Molekül, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, wobei die DNA-Sequenz SEQ ID Nr. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist.
2. Isoliertes infektiöses RNA-Molekül, das durch ein isoliertes Polynucleotid-Molekül nach Anspruch 1 codiert wird, wobei des infektiöse RNA-Molekül ein Nord-Amerikanisches PRRS-Virus codiert.
3. Isoliertes Polynucleotid-Molekül nach Anspruch 1, in Form eines Plasmids.
4. Transfizierte Wirtszelle, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, wobei die DNA-Sequenz SEQ ID Nr. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, wobei die transfizierte Wirtszelle das codierte Nord-Amerikanische PRRS-Virus exprimieren kann.
5. Plasmid, das eine geeignete Wirtszelle direkt transfizieren und ein Nord-Amerikanisches PRRS-Virus aus der so transfizierten geeigneten Wirtszelle exprimieren kann, wobei das Plasmid a) eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das das Nord-Amerikanische PRRS-Virus codiert, und wobei die DNA-Sequenz SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von

Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, und b) einen Promoter, der das infektiöse RNA-Molekül in der geeigneten Wirtszelle transkribieren kann.

6. Verfahren zur Erzeugung eines Nord-Amerikanischen PRRS-Virus, wobei das Verfahren das Transfizieren einer geeigneten Wirtszelle mit einem Plasmid nach Anspruch 5, das das Nord-Amerikanische PRRS-Virus codiert, und den Erhalt des Nord-Amerikanischen PRRS-Virus, der durch die transfizierte Wirtszelle erzeugt wurde, umfaßt.

7. Isoliertes Polynucleotid-Molekül, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, das genetisch so modifiziert wurde, daß es, wenn es ein vom Schwein stammendes Tier infiziert, kein PRRS in dem Tier erzeugen kann, wobei die DNA-Sequenz SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, außer für den Fall, daß sie eine oder mehrere Mutationen enthält, die das codierte PRRS-Virus genetisch daran hindern PRRS zu erzeugen.

8. Transfizierte Wirtszelle, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, das genetisch so modifiziert wurde, daß es, wenn es ein vom Schwein stammendes Tier infiziert, kein PRRS in dem Tier erzeugen kann, wobei die DNA-Sequenz SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, außer für den Fall, daß sie eine oder mehrere Mutationen enthält, die das codierte PRRS-Virus genetisch daran hindern PRRS zu erzeugen, wobei die transfizierte Wirtszelle das codierte, genetisch modifizierte Nord-Amerikanische PRRS-Virus exprimieren kann.

9. Impfstoff zum Schutz eines vom Schwein stammenden Tiers vor einer Infektion durch ein PRRS-Virus, wobei der Impfstoff ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus, codiert durch ein infektiöses RNA-Molekül, codiert durch ein Polynucleotid-Molekül nach Anspruch 7, oder das infektiöse RNA-Molekül oder das Polynucleotid-Molekül in Form eines Plasmids, oder einen viralen Vektor, umfassend eine Nucleotidsequenz, die Anspruch 7 entspricht, die ein Nord-Amerikanisches PRRS-Virus codiert, der eine wirksame immunschützende Reaktion gegen die Infektion durch ein PRRS-Virus auslösen kann, in einer Menge, die zur Erzeugung von Immunschutz gegen die Infektion durch ein PRRS-Virus wirksam ist; und einen Träger, der für die veterinäre Verwendung akzeptabel ist, umfaßt.

10. Isoliertes Polynucleotid-Molekül, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, das genetisch so modifiziert ist, daß es ein oder mehrere heterologe antigene Epitope umfaßt, wobei die DNA-Sequenz, die das RNA-Molekül codiert, das das Nord-Amerikanische PRRS-Virus codiert, SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, außer für den Fall, daß sie eine oder mehrere weitere Nucleotidsequenzen umfaßt, die jeweils ein heterologes antigenes Epitop codieren, und wobei jedes heterologe antigenes Epitop eine wirksame immunschützende Reaktion gegen ein bestimmtes Pathogen in einem Säuger oder einem Vogel induzieren kann.

11. Isoliertes Polynucleotid-Molekül nach Anspruch 10 in Form eines Plasmids.

12. Transfizierte Wirtszelle, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, das genetisch so modifiziert ist, daß es ein oder mehrere heterologe antigene Epitope umfaßt, wobei die DNA-Sequenz, die das RNA-Molekül codiert, das das Nord-Amerikanische PRRS-Virus codiert, SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, außer für den Fall, daß sie eine oder mehrere weitere Nucleotidsequenzen umfaßt, die jeweils ein heterologes antigenes Epitop codieren, und wobei jedes heterologe antigenes Epitop eine wirksame immunschützende Reaktion gegen ein bestimmtes Pathogen in einem Säuger oder einem Vogel induzieren kann.

13. Impfstoff zum Schutz eines Säugers oder eines Vogels vor einer Infektion durch ein Pathogen, wobei der Impfstoff ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus, codiert durch ein infektiöses RNA-Molekül, codiert durch ein Polynucleotid-Molekül nach Anspruch 10, oder das infektiöse RNA-Molekül, oder ein Plasmid nach Anspruch 11, in einer Menge, die zur Erzeugung von Immunschutz gegen die Infektion durch das Pathogen wirksam ist, aus dem das/die heterologe(n) antigene(n) Epitop oder Epitope davon oder **dadurch** codiert stammen; und einen Träger, der für die pharmazeutische oder veterinäre Verwendung akzeptabel ist, umfaßt.
14. Isoliertes Polynucleotid-Molekül nach Anspruch 10, wobei die DNA-Sequenz, die das infektiöse RNA-Molekül codiert, das das genetisch modifizierte Nord-Amerikanische PRRS-Virus codiert, eine oder mehrere weitere Mutationen umfaßt, durch die das codierte genetisch modifizierte Nord-Amerikanische PRRS-Virus kein PRRS in einem vom Schwein stammenden Tier erzeugen kann.
15. Isoliertes Polynucleotid-Molekül nach Anspruch 14 in Form eines Plasmids.
16. Impfstoff zum Schutz eines vom Schwein stammenden Tieres vor der Infektion durch ein PRRS-Virus und vor der Infektion durch ein anderes Schweinepathogen als ein Nord-Amerikanisches PRRS-Virus, wobei der Impfstoff ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus, codiert durch ein infektiöses RNA-Molekül, codiert durch ein Polynucleotid-Molekül nach Anspruch 14, oder das infektiöse RNA-Molekül, oder ein Plasmid nach Anspruch 15, in einer Menge, die zur Erzeugung von Immunschutz gegen die Infektion durch ein PRRS-Virus und gegen die Infektion durch das Pathogen, aus dem das/die heterologe(n) antigene(n) Epitop oder Epitope davon oder **dadurch** codiert stammen, wirksam ist; und einen Träger, der für die veterinäre Verwendung akzeptabel ist, umfaßt.
17. Isoliertes Polynucleotid-Molekül, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, das genetisch so modifiziert ist, daß es ein oder mehrere detektierbare heterologe antigene Epitope umfaßt, wobei die DNA-Sequenz, die das infektiöse RNA-Molekül codiert, die gleiche wie SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, außer für den Fall, daß sie eine oder mehrere weitere Nucleotidsequenzen umfaßt, die jeweils ein detektierbares heterologes antigenes Epitop codieren.
18. Impfstoff zum Schutz eines vom Schwein stammenden Tieres vor der Infektion durch einen PRRS-Virus, wobei der Impfstoff ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus, codiert durch ein infektiöses RNA-Molekül, codiert durch ein Polynucleotid-Molekül nach Anspruch 17, umfassend ein oder mehrere detektierbare heterologe antigene Epitope, behandelt oder weiter genetisch modifiziert, so daß es kein PRRS in einem vom Schwein stammenden Tier erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen einen PRRS-Virus bei dem vom Schwein stammenden Tier auslösen kann; oder das infektiöse RNA-Molekül, wobei das genetisch modifizierte Nord-Amerikanische PRRS-Virus, das **dadurch** codiert ist, das ein oder mehrere detektierbare heterologe antigene Epitope umfaßt, weitere genetische Modifikationen umfaßt, so daß es kein PRRS erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen ein PRRS-Virus auslösen kann; oder ein isoliertes Polynucleotid nach Anspruch 17 in Form eines Plasmids, wobei das genetisch modifizierte Nord-Amerikanische PRRS-Virus, das **dadurch** codiert ist, das ein oder mehrere detektierbare heterologe antigene Epitope umfaßt, weitere genetische Modifikationen umfaßt, so daß es kein PRRS erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen ein PRRS-Virus auslösen kann; oder einen viralen Vektor, der eine Nucleotidsequenz, die ein infektiöses RNA-Molekül codiert, das ein solches genetisch modifiziertes Nord-Amerikanisches PRRS-Virus codiert, umfaßt, wobei das genetisch modifizierte Nord-Amerikanische PRRS-Virus, das **dadurch** codiert ist, das ein oder mehrere detektierbare heterologe antigene Epitope umfaßt, weitere genetische Modifikationen umfaßt, so daß es kein PRRS erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen einen PRRS-Virus auslösen kann; in einer Menge, die zur Erzeugung von Immunschutz gegen die Infektion durch einen PRRS-Virus wirksam ist; und einen Träger, der für die veterinäre Verwendung akzeptabel ist, umfaßt.
19. Isoliertes Polynucleotid-Molekül, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Nord-Amerikanisches PRRS-Virus codiert, das genetisch so modifiziert ist, daß es ihm an einem detektierbaren antigenen Epitop fehlt, wobei die DNA-Sequenz SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, außer für den Fall, daß es ihm an einer oder mehreren DNA-Sequenzen fehlt, die ein detektierbares antigenes Epitop codieren.

20. Impfstoff zum Schutz eines vom Schwein stammenden Tieres vor der Infektion durch ein PRRS-Virus, wobei der Impfstoff ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus, codiert durch ein infektiöses RNA-Molekül, codiert durch ein Polynucleotid-Molekül nach Anspruch 19, dem es an einem oder mehreren detektierbaren antigenen Epitopen fehlt, behandelt oder weiter genetisch modifiziert, so daß es kein PRRS in einem vom Schwein stammenden Tier erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen ein PRRS-Virus bei einem vom Schwein stammenden Tier auslösen kann; oder das infektiöse RNA-Molekül, wobei das genetisch modifizierte Nord-Amerikanische PRRS-Virus, das **dadurch** codiert ist, dem es an einem oder mehreren detektierbaren antigenen Epitopen mangelt, weitere genetische Modifikationen umfaßt, so daß es kein PRRS erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen einen PRRS-Virus auslösen kann; oder ein isoliertes Polynucleotid-Molekül in Form eines Plasmids, wobei das genetisch modifizierte Nord-Amerikanische PRRS-Virus, das **dadurch** codiert ist, dem es an einem oder mehreren detektierbaren antigenen Epitopen mangelt, weitere genetische Modifikationen umfaßt, so daß es kein PRRS erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen einen PRRS-Virus auslösen kann; oder einen viralen Vektor, der eine Nucleotidsequenz, die ein infektiöses RNA-Molekül codiert, das ein solches genetisch modifiziertes Nord-Amerikanisches PRRS-Virus codiert, umfaßt, wobei das genetisch modifizierte Nord-Amerikanische PRRS-Virus, das **dadurch** codiert ist, dem es an einem oder mehreren detektierbaren antigenen Epitopen mangelt, weitere genetische Modifikationen umfaßt, so daß es kein PRRS erzeugen kann, aber trotzdem eine wirksame immunschützende Reaktion gegen einen PRRS-Virus auslösen kann; in einer Menge, die zur Erzeugung von Immunschutz gegen die Infektion durch einen PRRS-Virus wirksam ist; und einen Träger, der für die veterinäre Verwendung akzeptabel ist, umfaßt.
21. Isoliertes Polynucleotid-Molekül, umfassend eine oder mehrere Nucleotidsequenzen, die jeweils ein Peptid, codiert durch ein Nord-Amerikanisches PRRS-Virus, codieren, wobei die Genomsequenz des Nord-Amerikanischen PRRS-Virus die gleiche ist, wie ein RNA-Molekül, das SEQ ID NR. 1 oder der Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 entspricht, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist.
22. Transfizierte Wirtszelle, umfassend eine oder mehrere Nucleotidsequenzen, die jeweils ein Peptid, codiert durch ein Nord-Amerikanisches PRRS-Virus, codieren, wobei die Genomsequenz des Nord-Amerikanischen PRRS-Virus die gleiche ist, wie ein RNA-Molekül, das SEQ ID NR. 1 oder der Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 entspricht, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist, wobei die transformierte Zelle das Peptid oder die Peptide exprimieren kann.
23. Verfahren zur Herstellung eines genetisch modifizierten Nord-Amerikanischen PRRS-Virus, das eine immunschützende Reaktion in einem Säuger oder einem Vogel, die damit geimpft wurden, auslösen kann, wobei das Verfahren den Erhalt eines isolierten Polynucleotid-Moleküls, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein Wildtyp-Nord-Amerikanisches PRRS-Virus codiert, wobei die DNA-Sequenz SEQ ID NR. 1 oder die Sequenz, beginnend mit und einschließlich Nucleotid 1 bis und einschließlich Nucleotid 15.416 von SEQ ID Nr. 1 ist, außer daß das Nucleotid, das dem Nucleotid 12.622 von SEQ ID Nr. 1 entspricht, Guanin anstelle von Adenin ist, und das Nucleotid, das dem Nucleotid 1.559 von SEQ ID Nr. 1 entspricht, Thymin anstelle von Cytosin ist; die genetische Mutation der DNA-Sequenz, die das infektiöse RNA-Molekül codiert, das das Wildtyp-Nord-Amerikanische PRRS-Virus codiert, um so ein isoliertes Polynucleotid-Molekül zu erhalten, umfassend eine DNA-Sequenz, die ein infektiöses RNA-Molekül codiert, das ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus codiert, wobei das Virus weiterhin eine wirksame immunschützende Reaktion gegen die Infektion durch das Wildtyp-Nord-Amerikanische PRRS-Virus in einem Säuger oder einem Vogel auslösen kann; und das Exprimieren des genetisch modifizierten Nord-Amerikanischen PRRS-Virus von dem isolierten Polynucleotid-Molekül, das so erhalten wurde, umfaßt.
24. Impfstoff zum Schutz eines Säugers oder eines Vogels vor der Infektion durch ein Nord-Amerikanisches PRRS-Virus, das ein genetisch modifiziertes Nord-Amerikanisches PRRS-Virus gemäß Anspruch 23 in einer Menge, die für das Auslösen einer wirksamen immunschützenden Reaktion gegen das Wildtyp-Nord-Amerikanische PRRS-Virus in einem Säuger oder einem Vogel, der damit geimpft wurde, wirksam ist, und einen Träger, der für die pharmazeutische oder veterinäre Verwendung akzeptabel ist, umfaßt.

Revendications

1. Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord, dans laquelle ladite séquence d'ADN a la séquence SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine.
2. Molécule d'ARN infectieuse isolée et codée par une molécule polynucléotidique isolée suivant la revendication 1, molécule d'ARN infectieuse qui code pour un virus PRRS d'Amérique du Nord.
3. Molécule polynucléotidique isolée suivant la revendication 1, sous forme d'un plasmide.
4. Cellule hôte transfectée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord, dans laquelle ladite séquence d'ADN est la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, cellule hôte transfectée qui est capable d'exprimer le virus PRRS d'Amérique du Nord codé.
5. Plasmide capable de transfecter directement une cellule hôte convenable et d'exprimer un virus PRRS d'Amérique du Nord à partir de la cellule hôte convenable ainsi transfectée, plasmide qui comprend a) une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour le virus PRRS d'Amérique du Nord, ladite séquence d'ADN étant la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, et b) un promoteur capable de transcrire ladite molécule d'ARN infectieuse dans ladite cellule hôte convenable.
6. Procédé pour engendrer un virus PRRS d'Amérique du Nord, procédé qui comprend la transfection d'une cellule hôte convenable avec un plasmide suivant la revendication 5 codant pour le virus PRRS d'Amérique du Nord et l'obtention du virus PRRS d'Amérique du Nord engendré par la cellule hôte transfectée.
7. Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte que, lorsqu'il infecte un animal porcin, il est incapable de produire le PRRS chez l'animal, dans laquelle ladite séquence d'ADN est la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle contient une ou plusieurs mutations qui empêchent génétiquement le virus PRRS codé d'être apte à produire le PRRS.
8. Cellule hôte transfectée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte que, lorsqu'il infecte un animal porcin, il est incapable de produire le PRRS chez l'animal, dans laquelle ladite séquence d'ADN est la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle contient une ou plusieurs mutations qui empêchent génétiquement le virus PRRS codé d'être apte à produire le PRRS, cellule hôte transfectée qui est capable d'exprimer le virus PRRS d'Amérique du Nord modifié génétiquement codé.
9. Vaccin pour la protection d'un animal porcin contre l'infection par un virus PRRS, vaccin qui comprend un virus PRRS d'Amérique du Nord modifié génétiquement codé par une molécule d'ARN infectieuse codée par une molécule polynucléotidique suivant la revendication 7, ou bien ladite molécule d'ARN infectieuse, ou ladite molécule polynucléotidique sous forme d'un plasmide, ou un vecteur viral comprenant une séquence de nucléotides correspondant à la revendication 7 codant pour un virus PRRS d'Amérique du Nord qui est capable d'engendrer une réponse

immunoprotectrice efficace contre l'infection par un virus PRRS, en une quantité efficace pour produire une immunoprotection contre l'infection par un virus PRRS ; et un support acceptable pour une utilisation vétérinaire.

- 5 **10.** Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte qu'il comprenne un ou plusieurs épitopes antigéniques hétérologues, dans laquelle la séquence d'ADN codant pour la molécule d'ARN codant pour le virus PRRS d'Amérique du Nord est la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle comprend une ou plusieurs séquences de nucléotides supplémentaires qui codent chacune pour un épitope antigénique hétérologue, et chaque épitope antigénique hétérologue est capable d'induire une réponse immunoprotectrice efficace contre un agent pathogène particulier chez un mammifère ou un oiseau.
- 15 **11.** Molécule polynucléotidique isolée suivant la revendication 10, sous forme d'un plasmide.
- 20 **12.** Cellule hôte transfectée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte qu'il comprenne un ou plusieurs épitopes antigéniques hétérologues, dans laquelle la séquence d'ADN codant pour la molécule d'ARN codant pour le virus PRRS d'Amérique du Nord a la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle comprend une ou plusieurs séquences de nucléotides supplémentaires qui codent chacune pour un épitope antigénique hétérologue, et chaque épitope antigénique hétérologue est capable d'induire une réponse immunoprotectrice efficace contre un agent pathogène particulier chez un mammifère ou un oiseau.
- 25 **13.** Vaccin pour la protection d'un mammifère ou d'un oiseau contre l'infection par un agent pathogène, vaccin qui comprend un virus PRRS d'Amérique du Nord modifié génétiquement codé par une molécule d'ARN infectieuse codée par une molécule polynucléotidique de la revendication 10, ou bien ladite molécule d'ARN infectieuse, ou un plasmide suivant la revendication 11, en une quantité efficace pour produire une immunoprotection contre l'infection par l'agent pathogène duquel sont dérivés le ou les épitopes antigéniques hétérologues de celui-ci ou ainsi codés ; et un support acceptable pour une utilisation pharmaceutique ou vétérinaire.
- 30 **14.** Molécule polynucléotidique isolée suivant la revendication 10, dans laquelle la séquence d'ADN codant pour la molécule d'ARN infectieuse codant pour le virus PRRS d'Amérique du Nord modifié génétiquement comprend une ou plusieurs mutations supplémentaires qui empêchent le virus PRRS d'Amérique du Nord modifié génétiquement codé d'être apte à produire le PRRS chez un animal porcin.
- 35 **15.** Molécule polynucléotidique isolée suivant la revendication 14, sous forme d'un plasmide.
- 40 **16.** Vaccin pour la protection d'un animal porcin contre l'infection par un virus PRSS et contre l'infection par un agent pathogène porcin autre qu'un virus PRRS d'Amérique du Nord, vaccin qui comprend un virus PRRS d'Amérique du Nord modifié génétiquement codé par une molécule d'ARN infectieuse codée par une molécule polynucléotidique suivant la revendication 14, ou ladite molécule d'ARN infectieuse, ou bien un plasmide suivant la revendication 15, en une quantité efficace pour produire une immunoprotection contre l'infection par un virus PRRS et contre l'infection par l'agent pathogène duquel sont dérivés le ou les épitopes antigéniques hétérologues de celui-ci ou ainsi codés ; et un support acceptable pour une utilisation vétérinaire.
- 45 **17.** Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte qu'il comprenne un ou plusieurs épitopes antigéniques hétérologues détectables, dans laquelle la séquence d'ADN codant pour la molécule d'ARN infectieuse est identique à la séquence SEQ ID N°1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle comprend une ou plusieurs séquences de nucléotides supplémentaires qui codent chacune pour un épitope antigénique hétérologue détectable.
- 50 **17.** Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte qu'il comprenne un ou plusieurs épitopes antigéniques hétérologues détectables, dans laquelle la séquence d'ADN codant pour la molécule d'ARN infectieuse est identique à la séquence SEQ ID N°1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle comprend une ou plusieurs séquences de nucléotides supplémentaires qui codent chacune pour un épitope antigénique hétérologue détectable.
- 55 **17.** Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte qu'il comprenne un ou plusieurs épitopes antigéniques hétérologues détectables, dans laquelle la séquence d'ADN codant pour la molécule d'ARN infectieuse est identique à la séquence SEQ ID N°1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle comprend une ou plusieurs séquences de nucléotides supplémentaires qui codent chacune pour un épitope antigénique hétérologue détectable.

18. Vaccin pour la protection d'un animal porcin contre l'infection par un virus PRRS, vaccin qui comprend un virus PRRS d'Amérique du Nord modifié génétiquement codé par une molécule d'ARN infectieuse codée par une molécule polynucléotidique suivant la revendication 17, comprenant un ou plusieurs épitopes antigéniques hétérologues détectables, traité ou modifié en outre génétiquement de telle sorte qu'il soit incapable de produire le PRRS chez un animal porcin tout en étant capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS chez l'animal porcin ; ou bien ladite molécule d'ARN infectieuse, dans lequel le virus PRRS d'Amérique du Nord modifié génétiquement ainsi codé comprenant un ou plusieurs épitopes antigéniques hétérologues détectables comprend d'autres modifications génétiques de telle sorte qu'il soit incapable de produire le PRRS tout en étant capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS ; ou bien un polynucléotide isolé suivant la revendication 17, sous forme d'un plasmide, dans lequel le virus PRRS d'Amérique du Nord modifié génétiquement ainsi codé comprenant un ou plusieurs épitopes antigéniques hétérologues et détectables comprend des modifications génétiques supplémentaires de telle sorte qu'il soit incapable de produire le PRRS tout en étant capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS ; ou un vecteur viral comprenant une séquence de nucléotides codant pour une molécule d'ARN infectieuse codant pour un tel virus PRRS d'Amérique du Nord modifié génétiquement, dans lequel le virus PRRS d'Amérique du Nord modifié génétiquement ainsi codé comprenant un ou plusieurs épitopes antigéniques hétérologues détectables comprend des modifications génétiques supplémentaires de telle sorte qu'il soit incapable de produire le PRRS tout en étant encore capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS ; en une quantité efficace pour produire une immunoprotection contre l'infection par un virus PRRS ; et un support acceptable pour une utilisation vétérinaire.
19. Molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord qui est modifié génétiquement de telle sorte qu'il soit dépourvu d'épitope antigénique détectable, dans laquelle ladite séquence d'ADN a la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, sauf qu'elle est dépourvue d'une ou plusieurs séquences d'ADN codant pour un épitope antigénique détectable.
20. Vaccin pour la protection d'un animal porcin contre une infection par un virus PRRS, vaccin qui comprend un virus PRRS d'Amérique du Nord modifié génétiquement codé par une molécule d'ARN infectieuse codée par une molécule polynucléotidique suivant la revendication 19, dépourvu d'un ou plusieurs épitopes antigéniques détectables, traité ou modifié de plus génétiquement de telle sorte qu'il soit incapable de produire le PRRS chez un animal porcin tout en étant encore capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS chez l'animal porcin ; ou ladite molécule d'ARN infectieuse, dans lequel le virus PRRS d'Amérique du Nord modifié génétiquement ainsi codé qui est dépourvu d'un ou plusieurs épitopes antigéniques détectables comprend des modifications génétiques supplémentaires de telle sorte qu'il soit incapable de produire le PRRS tout en étant encore capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS ; ou une molécule polynucléotidique isolée, sous forme d'un plasmide, dans lequel le virus PRRS d'Amérique du Nord modifié génétiquement ainsi codé qui est dépourvu d'un ou plusieurs épitopes antigéniques détectables comprend des modifications génétiques supplémentaires de telle sorte qu'il soit incapable de produire le PRRS tout en étant encore capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS ; ou un vecteur viral comprenant une séquence de nucléotides codant pour une molécule d'ARN infectieuse codant pour un tel virus PRRS d'Amérique du Nord modifié génétiquement, dans lequel le virus PRRS d'Amérique du Nord modifié génétiquement ainsi codé qui est dépourvu d'un ou plusieurs épitopes antigéniques détectables comprend des modifications génétiques supplémentaires de telle sorte qu'il soit incapable de produire le PRRS tout en étant encore capable d'engendrer une réponse immunoprotectrice efficace contre un virus PRRS ; en une quantité efficace pour produire une immunoprotection contre l'infection par un virus PRRS ; et un support acceptable pour une utilisation vétérinaire.
21. Molécule polynucléotidique isolée comprenant une ou plusieurs séquences de nucléotides qui codent chacune pour un peptide codé par un virus PRRS d'Amérique du Nord, dans laquelle la séquence génomique dudit virus PRRS d'Amérique du Nord est identique à une molécule d'ARN correspondant à la SEQ ID n° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine.
22. Cellule hôte transfectée comprenant une ou plusieurs séquences de nucléotides qui codent chacune pour un peptide codé par un virus PRRS d'Amérique du Nord, dans laquelle la séquence génomique dudit virus PRRS d'Amérique du Nord est identique à une molécule d'ARN correspondant à la SEQ ID N° 1 ou la séquence débutant par et

comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, cellule transformée qui est capable d'exprimer ledit ou lesdits peptides.

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23. Procédé pour la préparation d'un virus PRRS d'Amérique du Nord modifié génétiquement qui est capable d'engendrer une réponse immunoprotectrice chez un mammifère ou un oiseau vacciné avec celui-ci, procédé qui comprend l'obtention d'une molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord de type sauvage, dans lequel ladite séquence d'ADN a la SEQ ID N° 1 ou la séquence débutant par et comprenant le nucléotide 1 jusqu'au et y compris le nucléotide 15 416 de la SEQ ID N° 1, sauf que le nucléotide correspondant au nucléotide 12 622 de la SEQ ID N° 1 est un nucléotide à guanine au lieu d'une adénine et le nucléotide correspondant au nucléotide 1 559 de la SEQ ID N° 1 est un nucléotide à thymine au lieu d'une cytosine, la mutation génétique de la séquence d'ADN codant pour la molécule d'ARN infectieuse codant pour le virus PRRS d'Amérique du Nord de type sauvage de manière à obtenir une molécule polynucléotidique isolée comprenant une séquence d'ADN codant pour une molécule d'ARN infectieuse codant pour un virus PRRS d'Amérique du Nord modifié génétiquement, virus qui reste capable d'engendrer une réponse immunoprotectrice efficace contre une infection par le virus PRRS d'Amérique du Nord de type sauvage chez un mammifère ou un oiseau, et l'expression du virus PRRS d'Amérique du Nord modifié génétiquement à partir de la molécule polynucléotidique isolée ainsi obtenue.
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24. Vaccin pour la protection d'un mammifère ou d'un oiseau contre l'infection par un virus PRRS d'Amérique du Nord, qui comprend un virus PRRS d'Amérique du Nord modifié génétiquement suivant la revendication 23 en une quantité efficace pour engendrer une réponse immunoprotectrice efficace contre un virus PRRS d'Amérique du Nord de type sauvage chez un mammifère ou un oiseau vacciné avec celui-ci, et un support acceptable pour une utilisation pharmaceutique ou vétérinaire.
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FIG. 1

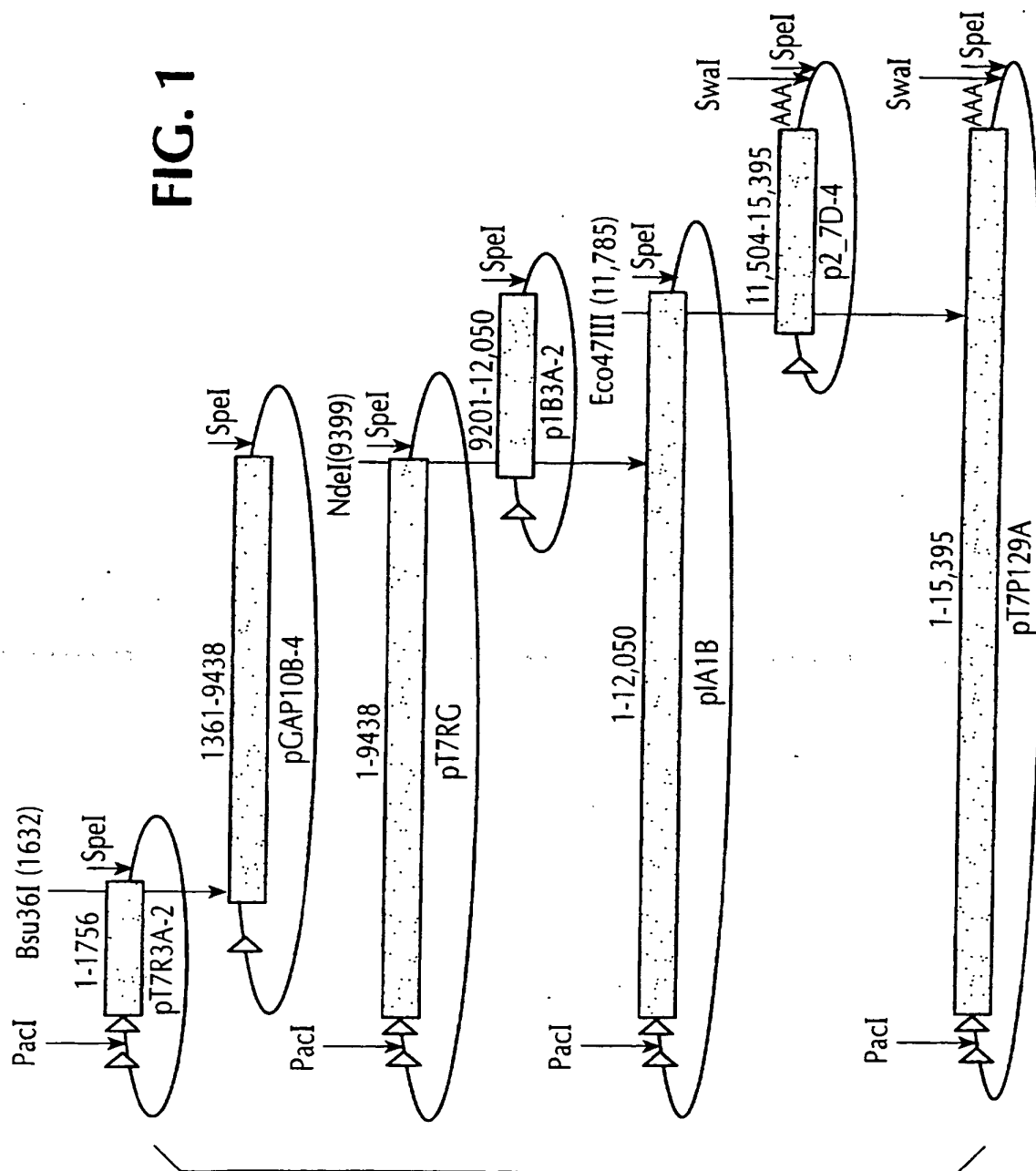
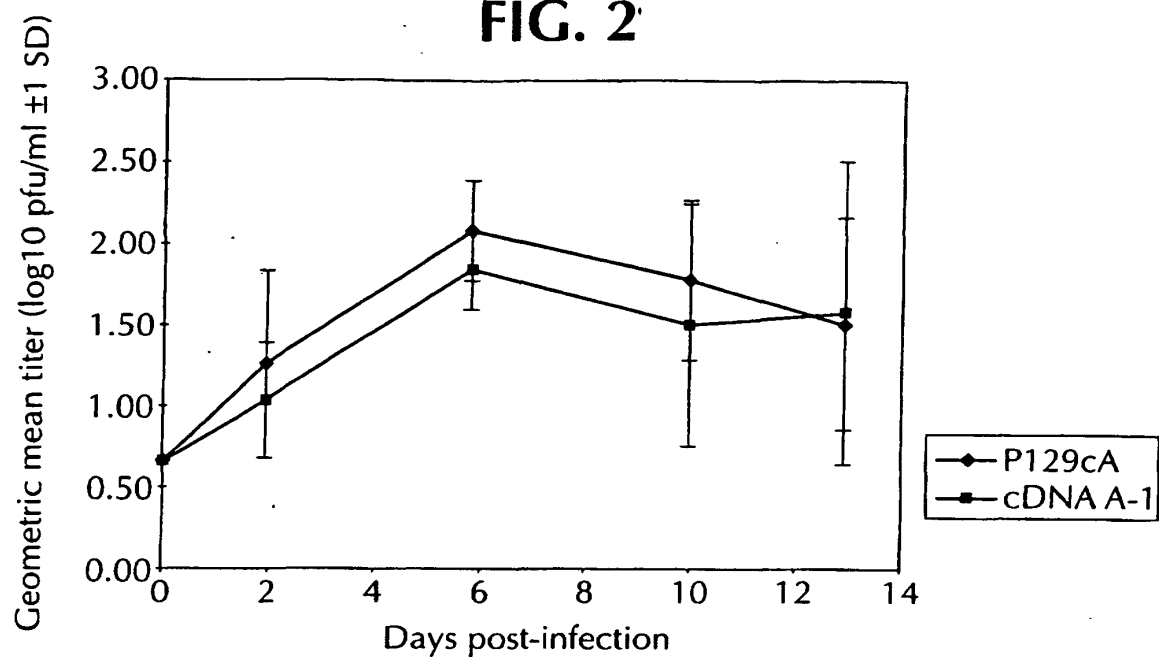


FIG. 2**FIG. 3**