

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2017378336 B2**

(54) Title
Effective vaccination against European strains of porcine reproductive and respiratory syndrome (PRRS) virus prior to weaning

(51) International Patent Classification(s)
A61K 39/12 (2006.01) **C12N 7/00** (2006.01)

(21) Application No: **2017378336** (22) Date of Filing: **2017.12.14**

(87) WIPO No: **WO18/112169**

(30) Priority Data

(31)	Number	(32)	Date	(33)	Country
	62/434,144		2016.12.14		US

(43) Publication Date: **2018.06.21**

(44) Accepted Journal Date: **2024.06.06**

(71) Applicant(s)
Zoetis Services LLC

(72) Inventor(s)
Calvert, Jay Gregory;Balasch, Monica;Fort, Maria;Pearce, Douglas S.;Keith, Marcia L.

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

(56) Related Art
WO 2016/012406 A2
WO 2012/110489 A2
WO 2013/173443 A1
CALVERT JAY G, et al., "CD163 expression confers susceptibility to porcine reproductive and respiratory syndrome viruses," Journal of Virology, (2007), vol 81 no. 14, pp 7371-7379.



(51) International Patent Classification:

A61K 39/12 (2006.01) C12N 7/00 (2006.01)

(21) International Application Number:

PCT/US2017/066347

(22) International Filing Date:

14 December 2017 (14.12.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/434,144 14 December 2016 (14.12.2016) US

(71) Applicant: ZOETIS SERVICES LLC [US/US]; 10 Sylvan Way, Parsippany, NJ 07054 (US).

(72) Inventors: CALVERT, Jay, Gregory; c/o Zoetis Services LLC, 333 Portage Street, Kalamazoo, MI 49007 (US). BALASCH, Monica; c/o Zoetis Services LLC, 10 Sylvan Way, Parsippany, NJ 07054 (US). FORT, Maria; c/o Zoetis Services LLC, 10 Sylvan Way, Parsippany, NJ 07054 (US). PEARCE, Douglas, S.; c/o Zoetis Services LLC, 333 Portage Street, Kalamazoo, MI 49007 (US). KEITH, Marcia, L.; c/o Zoetis Services LLC, 333 Portage Street, Kalamazoo, MI 49007 (US).

(74) Agent: DONAHUE, Victor E. et al.; Zoetis Services LLC, 10 Sylvan Way, Parsippany, NJ 07054 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:

26 July 2018 (26.07.2018)

(54) Title: EFFECTIVE VACCINATION AGAINST EUROPEAN STRAINS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS PRIOR TO WEANING

(57) Abstract: The present invention provides modified live European PRRS viruses (whether or not recombinant) that have been at all times cultured, maintained, and/or attenuated on non-simian cells, typically porcine cells, wherein such cells express a porcine CD163 receptor, and whereinby vaccines prepared from such viruses are safe and efficacious, and permit the pre-weaning single dose vaccination of swine, typically as early as Day 1 of life.



EFFECTIVE VACCINATION AGAINST EUROPEAN STRAINS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS PRIOR TO WEANING

5 FIELD OF THE INVENTION

The present invention provides vaccines that protect swine of all ages, both male and female, against European strains of porcine reproductive and respiratory syndrome (PRRS) virus. The present invention particularly provides for the safe and early vaccination of piglets prior to weaning, including from immediately after birth (i.e. only about 1 day of age or less) to three weeks of age, at all times optionally in combination with multivalent combination swine vaccines, such as bivalent PRRSV/*Mycoplasma hyopneumoniae* (*M.hyo*) vaccines, bivalent PRRSV/Porcine Circovirus type 2 (PCV2) vaccines, and trivalent PRRSV/*M.hyo*/PCV2 vaccines, or simply as a monovalent PRRSV vaccine, wherein the PRRS vaccine component generally corresponds to a genotype thereof (and sub-types of said genotype) recognized as circulating on the European continent, to include both Western Europe, Mediterranean regions, Scandinavian regions, and Eastern Europe such as Russia, Turkey, and the Ukraine.

The present invention is broadly directed to novel classes of attenuated European PRRS strains which, in modified live form, are both remarkably safe and effective when given even to the youngest animals as vaccines, and provide very long duration of immunity.

BACKGROUND OF THE INVENTION

Porcine reproductive and respiratory syndrome (PRRS) is characterized by abortions, stillbirths, and other reproductive problems in sows and gilts, as well as respiratory disease in young pigs. The causative agent is the PRRS virus (PRRSV), a member of the family *Arteriviridae* and the order *Nidovirales*. 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 non-structural proteins are translated directly from the genomic RNA of nidoviruses as large polyproteins and subsequently cleaved by viral proteases into discrete functional proteins. A 3'-coterminal nested set of subgenomic RNAs (sgRNAs) is synthesized from the genome and are used as messenger RNAs for translation of the

structural proteins. The reproduction of nidoviral genomic RNA is thus a combined process
35 of genome replication and sgRNA synthesis.

In the late 1980's, two distinct genotypes of the virus emerged nearly simultaneously, one in North America and another in Europe. PRRS virus is now endemic in nearly all swine producing countries, and is considered one of the most economically important diseases affecting the global pork industry. Additionally, highly virulent genotypes
40 have been isolated in China and surrounding countries, and such genotypes are generally closely related to North American genotypes.

Despite significant advances in understanding the biology of PRRSV, control of the virus remains difficult. Vaccination of animals in the field has proven to be largely ineffective. PRRS commonly re-emerges in immunized herds, and most on-farm PRRSV
45 vaccination campaigns ultimately fail to control the disease.

Without being limited as to theory, infection of pigs with wild type PRRSV or their vaccination with a live attenuated form of this pathogen unfortunately only elicits an exuberant production of non-neutralizing antibodies. During this time interval, for example, only limited quantities of interferon (IFN)- γ secreting cells are generated. Thus, PRRSV
50 seems to inherently stimulate an imbalanced immune response distinguished by consistently abundant humoral (antibody-based) immunity, and a variable and limited but potentially protective T helper (Th) 1-like IFN- γ response. One characteristic of PRRSV infection that most likely contributes to the imbalanced development of adaptive immunity is the lack of an adequate innate immune response. Usually, virus-infected cells secrete type I
55 interferon "IFN" (including IFN- α and IFN- β), which protects neighboring cells from infection. In addition, the released type I IFN interacts with a subset of naïve T cells to promote their conversion into virus-specific type II IFN (IFN- γ) secreting cells. In contrast, the IFN- α response of pigs to PRRSV exposure is nearly non-existent. Such inefficient stimulation of IFN- α production by a pathogen would be expected to have a significant impact on the
60 nature of the host's adaptive immune response, since IFN- α up-regulates IFN- γ gene expression. Accordingly, the former cytokine likely controls the dominant pathway that promotes the development of adaptive immunity, namely, T cell-mediated IFN- γ responses and peak antiviral immune defenses.

In this regard, it has become evident that a probable link between innate and
65 adaptive immunity in viral infections occurs through a special type of dendritic cell which has the ability to produce large amounts of type I interferon, and which plays a critical role in the

polarization of T-cell function. Specifically, an infrequent but remarkable type of dendritic cell, the plasmacytoid dendritic cell (PDC), also known as a natural IFN- α/β -producing cell, plays a critical role in anti-viral immunity by means of their ability to cause naïve T cells to differentiate into IFN- γ secreting cells. Although rare, the PDC are enormously potent producers of IFN- α , with each cell being capable of producing 3-10 pg of IFN- α in response to virus. In contrast, monocytes produce 5- to 10-fold less IFN- α on a per cell basis. The phenotype and some biological properties of porcine PDC have been described (Summerfield *et al.*, 2003, Immunology 110:440). Recent studies have determined that PRRSV does not stimulate porcine PDCs to secrete IFN- α (Calzada *et al.*, 2010, Veterinary Immunology and Immunopathology 135:20).

This fact, in combination with the observation that exogenously added IFN- α at the time of vaccination has been found to improve the intensity of the PRRSV-specific IFN- γ response (W.A. Meier et al., Vet. Immunol. Immunopath. 102, pp 299-314, 2004), highlights the critical role that IFN- α plays during the infection of pigs with this virus. Given the apparent critical role of IFN- α on the development of protective immunity, it is important to determine the ability of different PRRS virus stocks to stimulate and/or inhibit the production of IFN- α . Accordingly, there is a pressing need for new and improved modified live vaccines to protect against PRRS.

European PRRS is generally denominated 'type 1' to distinguish it from distantly related North American or "type 2" PRRS, and the two types are only about 60% identical, on a total nucleotide level. The first clearly defined European PRRSV isolate was disclosed by Wensvoort et al., as the Lelystad agent (see Institut Pasteur Isolate 1102, and U.S. Patents 5,620,691 and 6,197,310 and their international counterparts). The various subtypes of European PRRS (all of which can be protected against in all aspects of the present invention) are further elaborated in M. P. Murtaugh et al., Virus Research, Vol 154, pp. 18-30, 2010; and M. Shi et al., Virus Research, vol 154 pp. 7-17, 2010. Early North American PRRS isolates are disclosed in U.S. Patents 5,476,778 and 5,840, 563.

The present inventors have discovered that it is possible to provide attenuated forms of European PRRS strains that prove both safe and effective when given to the youngest of animals, and that unlike many other currently available PRRS vaccines, do not disable many aspects of the animal's immune response mechanisms, such as those aforementioned, thereby further contributing to safety and efficacy. As is well known in the art, it has proved very difficult to culture PRRS virus. Historically, monkey kidney cell line

100 MA-104 and its derivatives have been used almost exclusively for the propagation and
attenuation of commercial modified live PRRSV vaccines, although it was not known why
such cells were operable for this purpose (see U.S. Patent 5,476,778).

The discovery that the mammalian cell surface protein "CD163" is naturally used
by PRRS virus to enter mammalian cells (see for example US Patent 9,102,912) has
105 permitted the development of new PRRSV-permissive cell lines. Surprisingly, attenuation on
these newer cell lines yields vaccine viruses with properties that differ from existing
vaccines (see U.S. Patent 9,566,324 and U.S. application publication 2013-0309263)

Accordingly, it has been surprisingly discovered that European PRRS viruses
adapted to culture on either (1) porcine cells or (2) non-porcine cells that incorporate the
110 porcine CD163 receptor (see U. S. Patent 7,754,464) retain a safety and efficacy profile that
is more meaningful to porcine animals than that provided by vaccine viruses cultured by
traditional methods, and further that protective immunity can be achieved even with early
age vaccinations. It thus appears that the routine adaptation of PRRS to culture in monkey
kidney cell line MA-104 (which oddly expresses simian CD163 possibly in relation to a
115 hemoglobin scavenging function, and wherein said simian CD163 is only about 85%
identical to porcine CD163) leads to a safety/efficacy profile that will always be less
biologically relevant and non optimal. Representative examples of porcine CD163 are
described in U.S. Patent 7,754,464 and its related family members, U.S Patents 8,058,050;
8,486,685; and 9,102,912 (see, for example, SEQ ID: NO:14 and SEQ ID NO:2 therein).
120 As further described in this patent family, as applicable to the practice of the present
invention, it is not necessary for the recombinant expression cell to express full length
CD163, as long as the CD163 polypeptide has a transmembrane domain to anchor in the
membrane and expresses its surface exposed domains, thus the C-terminal domain may be
absent or reduced.

125 Additionally, vaccines of the present invention are expected to not downregulate
host interferon response, whereas prior art vaccines such as Ingelvac MLV (see US
5,476,778 and 5,846,805) that are adapted to culture in simian cells (that provide only
simian CD163 as receptor) generally show strong inhibitory effect on host IFN-alpha
response (see Example 5 generally, and Figure 4 in U.S. 9,566,324).

130 PRRS virus normally generates the most severe symptoms in the youngest of
animals, and being able to provide a vaccination that is both safe and efficacious to a piglet
that is only one day old, for example, substantially contributes to husbandry in the industry,

not only by decreasing mortality in young piglets, but also permitting the movement of animals between facilities at the earliest possible time post-weaning.

A further aspect of the invention provides the recognition that current European simian cell-based vaccines can be improved by re-adapting the underlying viruses either to grow in appropriate porcine cells (see discussion, for example, of PK-9 cells below) or non-porcine cells that express porcine CD163, with the result that the vaccines are generally safer, and can safely elicit a robust and enduring immune response at a much earlier age. Thus, the typical safety and efficacy requirements associated with traditional vaccines that have been prepared in simian cells (i.e. that such vaccines be given at only about 3 weeks of age or later) can be obviated with improved performance, also achieving effectiveness as early as day 1 of life. Thus, given the wide variety of types, subtypes and strains of PRRSV, the methods of the present invention can also be used to improve the current relevance and effectiveness of many preexisting live PRRS vaccines.

That Type 2 (North American) PRRS vaccines can be safe and effective when administered pre-weaning has been earlier reported in regard of North American/Asian strains of PRRS (see WO2013/173443 at Examples 9 and 10, for example), particularly referencing attenuates of the "P129 strain" of North American PRRS virus (see SEQ ID NO:6 therein, for example, and also PCT/IB2011/055003 and US 6,500,662). However, European and North American PRRS viruses are only about 60% identical at a nucleotide level, and behave in somewhat different ways, as to the types of pathologies seen, in different classes of animals (age, sex, and the like).

Here we describe the use of porcine CD163-expressing cell lines to attenuate and select Genotype 1 (European) PRRSV strains having excellent properties as vaccines.

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of the common general knowledge in the field.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a vaccine when used in protecting a porcine animal against infection by a PRRS virus, which vaccine comprises: (a) a European PRRS virus encoded by the polynucleotide molecule of SEQ ID NO: 5 (b) a polynucleotide molecule that is at least 90% identical to SEQ ID NO: 5 comprising mutations compared to SEQ ID NO: 1 selected from:

in the ORF1a amino acid sequence, S or a conservative substitution thereof at amino acid position 19; Y or a conservative substitution thereof at amino acid position 24; A or a conservative substitution thereof at amino acid position 156; Y or a conservative substitution thereof at amino acid position 157; D or a conservative substitution thereof at amino acid position 268; H or a conservative substitution thereof at amino acid position 294; Y or a conservative substitution thereof at amino acid position 416; S or a conservative substitution thereof at amino acid position 742; L or a conservative substitution thereof at amino acid position 884; P or a conservative substitution thereof at amino acid position 908; K or a conservative substitution thereof at amino acid position 916; K or a conservative substitution thereof at amino acid position 977; S or a conservative substitution thereof at amino acid position 1138; F or a conservative substitution thereof at amino acid position 1160; S or a conservative substitution thereof at amino acid position 1500; R or a conservative substitution thereof at amino acid position 2094; P or a conservative substitution thereof at amino acid position 2254; and L or a conservative substitution thereof at amino acid position 2290;

in the ORF1b amino acid sequence, S or a conservative substitution thereof at amino acid position 567; and H or a conservative substitution thereof at amino acid position 912;

in the ORF2a amino acid sequence, L or a conservative substitution thereof at amino acid position 22; F or a conservative substitution thereof at amino acid position 88; M or a conservative substitution thereof at amino acid position 94; and F or a conservative substitution thereof at amino acid position 95;

in the ORF2b amino acid sequence, L or a conservative substitution thereof at amino acid position 47;

in the ORF3 amino acid sequence, S or a conservative substitution thereof at amino acid position 52;

in the ORF4 amino acid sequence, T or a conservative substitution thereof at amino acid position 151;

in the ORF5 amino acid sequence, F or a conservative substitution thereof at amino acid position 20; and D or a conservative substitution thereof at amino acid position 37;

in the ORF5a amino acid sequence, V or a conservative substitution thereof at amino acid position 18; and R or a conservative substitution thereof at amino acid position 35; (c) said encoding polynucleotide molecule; (d) said polynucleotide molecule in the form of a plasmid; or (e) a viral vector comprising said polynucleotide molecule;

in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use, and wherein said use of the vaccine provides early and safe vaccination of piglets prior to weaning including from one day old or less to three weeks of age, and wherein said use of the vaccine provides a duration of immunity for up to 6 months.

In one aspect, the invention provides a use of a vaccine for the manufacture of a medicament for protecting a porcine animal against infection by a PRRS virus, which vaccine comprises: (a) a European PRRS virus encoded by the polynucleotide molecule of SEQ ID NO: 5 (b) a polynucleotide molecule that is at least 90% identical to SEQ ID NO: 5 comprising mutations compared to SEQ ID NO: 1 selected from:

in the ORF1a amino acid sequence, S or a conservative substitution thereof at amino acid position 19; Y or a conservative substitution thereof at amino acid position 24; A or a conservative substitution thereof at amino acid position 156; Y or a conservative substitution thereof at amino acid position 157; D or a conservative substitution thereof at amino acid position 268; H or a conservative substitution thereof at amino acid position 294; Y or a conservative substitution thereof at amino acid position 416; S or a conservative substitution thereof at amino acid position 742; L or a conservative substitution thereof at amino acid position 884; P or a conservative substitution thereof at amino acid position 908; K or a conservative substitution thereof at amino acid position 916; K or a conservative substitution thereof at amino acid position 977; S or a conservative substitution thereof at amino acid position 1138; F or a conservative substitution thereof at amino acid position 1160; S or a conservative substitution thereof at amino acid position 1500; R or a conservative substitution thereof at amino acid position 2094;

P or a conservative substitution thereof at amino acid position 2254; and L or a conservative substitution thereof at amino acid position 2290;

5 in the ORF1b amino acid sequence, S or a conservative substitution thereof at amino acid position 567; and H or a conservative substitution thereof at amino acid position 912;

in the ORF2a amino acid sequence, L or a conservative substitution thereof at amino acid position 22; F or a conservative substitution thereof at amino acid position 88; M or a conservative substitution thereof at amino acid position 94; and F or a conservative substitution thereof at amino acid position 95;

10 in the ORF2b amino acid sequence, L or a conservative substitution thereof at amino acid position 47;

in the ORF3 amino acid sequence, S or a conservative substitution thereof at amino acid position 52;

15 in the ORF4 amino acid sequence, T or a conservative substitution thereof at amino acid position 151;

in the ORF5 amino acid sequence, F or a conservative substitution thereof at amino acid position 20; and D or a conservative substitution thereof at amino acid position 37;

20 in the ORF5a amino acid sequence, V or a conservative substitution thereof at amino acid position 18; and R or a conservative substitution thereof at amino acid position 35; (c) said encoding polynucleotide molecule; (d) said polynucleotide molecule in the form of a plasmid; or (e) a viral vector comprising said polynucleotide molecule;

25 wherein said medicament is to be used in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use, and wherein said use of the vaccine provides early and safe vaccination of piglets prior to weaning including from one day old or less to three weeks of age, and wherein said use of the vaccine provides a duration of immunity for up to 6 months.

In a first embodiment, the present invention provides an isolated polynucleotide molecule including a DNA sequence encoding an infectious RNA molecule encoding a PRRS virus that is genetically modified such that, as a vaccine, it elicits an effective immunoprotective response against the PRRS virus in porcine animals. In certain aspects, the invention provides for a DNA sequence as set forth herein including SEQ ID NO.:5. Encoding sequences that are at least 80% identical to SEQ ID NO:5 (and more preferably having 85%, 90%, 95%, 96%, 97%, 98% or 99% identity thereto) are also operable in the practice of the present invention, as long as they provide various key mutations, as described below, that distinguish said isolates from other or precursor strains.

A further embodiment of the invention includes the RNA sequences corresponding to the aforementioned DNA polynucleotides, and that can be expressed therefrom, and the actual viruses that can be assembled from such expression. Additional attenuated European PRRS viruses useful in the practice of the invention include those that
170 are encoded by a DNA sequence that hybridizes to the complement of a DNA sequence of SEQ ID NO:5 under highly stringent conditions which comprise hybridization to filter bound DNA in 0.5 M NaHPO₄, 7% SDS, 1mM EDTA at 65 degrees C, and washing in 0.1 SSC/0/1%SDS at 68 degrees C.

For the purposes of the invention, "corresponding" means that the relative
175 sequences can be optimally aligned using a BLOSUM algorithm as described in Henikoff et al. Proc Natl. Acad. Sci., USA, 89, pp. 10915-10919, 1992.

In certain embodiments, the invention provides for a plasmid that includes an isolated polynucleotide molecule as set forth herein and a promoter capable of transcribing the polynucleotide molecule in a suitable host cell. In another embodiment, the European
180 PRRS encoding sequence of the plasmid herein further encodes one or more detectable heterologous antigenic epitopes. The present invention provides for a transfected host cell that includes the plasmid set forth herein.

In another aspect, the present invention provides for a vaccine for protecting a porcine animal from infection by a PRRS virus. The vaccine may include a European PRRS
185 virus encoded by an infectious RNA molecule, the infectious RNA molecule, each of which is encoded by the isolated polynucleotide molecule as set forth herein. In yet another aspect, the vaccine includes a plasmid or viral vector including the polynucleotide herein. The vaccine set forth herein may optionally include a vaccine carrier acceptable for veterinary use. In one important aspect, the vaccine has a decreased interferon- α inhibitory
190 effect as compared to wild-type European viruses and other available vaccines.

In one embodiment, the present invention provides for diagnostic kit including polynucleotide molecules which distinguish (a so-called DIVA test) between porcine animals naturally infected with a field strain of a PRRS virus and porcine animals vaccinated with the modified live vaccine set forth herein.

195 In other embodiments, the invention provides for a method of protecting a porcine animal from infection with a strain of PRRS virus, including administering to the animal an immunogenically protective amount of the vaccine of the claims set forth herein.

The present invention also provides a plasmid capable of directly transfecting a suitable host cell and expressing a Porcine Reproductive and Respiratory Syndrome Virus (PRRS) from the suitable host cell so transfected, which plasmid comprises: (a) a DNA sequence encoding an infectious RNA molecule encoding the PRRS virus, and (b) a promoter capable of transcribing said infectious RNA molecule.

In further preferred embodiments, said plasmid contains a promoter that is a eukaryotic promoter capable of permitting a DNA launch in targeted eukaryotic cells, or a prokaryotic or phage promoter capable of directing in vitro transcription of the plasmid. The invention similarly provides a method of generating a PRRS virus, which method comprises transfecting a suitable host cell with an appropriate plasmid and obtaining PRRS virus generated by the transfected cell.

The invention also provides for host cells transfected with polynucleotide molecules and provides vaccines for protecting a porcine animal against infection by a PRRS virus, which vaccine comprises: (a) a genetically modified European PRRS virus encoded by such aforementioned polynucleotide molecules, or (b) said infectious molecule, or (c) said polynucleotide molecule in the form of a plasmid, or (d) a viral vector comprising said polynucleotide molecule, wherein the PRRS virus is able to elicit an effective immunoprotective response against infection by PRRS virus, in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use.

Particularly, the invention includes a method for generating a European Porcine Reproductive and Respiratory Syndrome (PRRS) virus, which method comprises transfecting a suitable eucaryotic host cell with a composition selected from the group consisting of :

- (a) a plasmid comprising (1) a DNA sequence encoding an infectious RNA molecule encoding a European PRRS virus, wherein said encoding DNA sequence is SEQ ID: NO:5, or an encoding DNA sequence that is at least 85% identical to SEQ ID NO:5, and (2) a promoter capable of driving transcription of said DNA sequence encoding said infectious RNA molecule in said suitable host cell; and
- (b) an infectious PRRS RNA molecule produced from in vitro transcription of a plasmid comprising a DNA sequence encoding said infectious PRRS RNA molecule encoding a European PRRS virus, wherein said encoding DNA sequence is SEQ ID: NO:5, or an encoding DNA sequence that is at least 85% identical to SEQ ID NO:5,

wherein, for cases (a) and (b) an encoding sequence is used that is at least 85% identical to SEQ ID NO:5 and contains one or more particular mutations compared to a reference sequence.

235

In preferred examples, both protective and safe vaccination may be provided to the piglet (whether male or female) in a single dose at less than one day of age, such as 8-16 hours after birth, out to two weeks of age, or three weeks of age, taking into account that the piglet may be weaned at any point from about two to three weeks of age. Thus the present invention is generally applicable to single-dose early vaccination, meaning at any time on the first day of life (from birth to 24 hours of age), similarly on Day 2, Day 3, Day 4, Day 5, Day 6, Day 7, Days 1-10, Days 1-14, and Days 1-21, or simply vaccination that is conducted earlier than approximately the day of weaning. Two or more doses can optionally be used.

245

Early vaccination (typically by the intramuscular or intranasal route) against porcine reproductive and respiratory syndrome virus (PRRSV) under such conditions also provides an early onset of protective immunity, such as by about one to eight weeks of age; or between about two to six weeks following vaccination; and commonly by about 28 days following vaccination.

250

Vaccination according to the practice of the invention typically provides a duration of immunity to the piglet that is about 23-28 weeks, typically at least about 26 weeks, which is the full expected life of a market pig. Thus vaccine programs made possible by the present invention protect piglets when they are most vulnerable to infection and are most easily handled. Such vaccination also permits onset of immunity to coincide with when weaned piglets are removed from the sow, and may be transported to other facilities where further risk of infection by PRRSV may be present. The vaccines of the invention are also highly effective in protecting boars; and sows and gilts, thus indirectly protecting unborn offspring. Although intramuscular and intranasal vaccination is preferably contemplated, other routes are also applicable.

260

In a further example of the invention, although it is less preferred, the CD163 polypeptide expressed by the recombinant cells in which the European PRRS virus is cultured (and thus pre-adapted to infection and growth in porcine animals) is not porcine CD163, but rather is a mammalian CD163 having an amino acid sequence that is relatively close to the porcine sequence, such as at least 90% identical to SEQ ID NOS 2 or 14 of

265

U.S. 7,754,464. More preferably, this amino acid identity, 92%, 95%, 96%, 97%, 98% or 99%.

270 The invention also provides diagnostic kits comprising polynucleotide molecules which distinguish between porcine animals naturally infected with a field strain of a PRRS virus and porcine animals vaccinated with the vaccines of the invention, which vaccines (viruses) preferably evidence a decreased interferon- α inhibitory effect as compared to wild-type.

275

The invention further provides representative whole genome sequences of PRRSV derived from European (Belgian) PRRSV isolate 96V198. Isolate 96V198 itself was obtained in 1996 by Professor Hans Nauwynck, Ghent University, Belgium, from the lungs of a young pig with respiratory signs (Passage 0 was thus recovered from the serum of an infected pig (SEQ ID NO:1).

280

Passage 1 (SEQ ID NO:2) is the result of a single passage on primary porcine alveolar macrophages (PAMs). All subsequent passages were performed on the BHK21-C12-26 cell line. Passage 9 (SEQ ID NO:3) is immediately prior to biological cloning of the virus by three rounds of limiting dilution. Passage 14 (SEQ ID NO:4) is clone 1 (of six clones examined and evaluated). Clone 1 continued to become Master Seed Virus at passage 44, and MSV+5 passages became Suvaxyn® PRRS MLV product at passage 49 (SEQ ID NO:5). All genomes are 15,092 nt long. All genomes are at least 99.5% identical to each other, and are about 92% identical to Lelystad virus.

285

BRIEF DESCRIPTION OF THE FIGURES

290 Figure 1 provides the full length nucleotide sequence (15,092 bases) of the passage 49 isolate for the Suvaxyn® PRRS MLV product, SEQ ID NO:5.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 provides passage 0 of PRRSV isolate 96V198.

295 SEQ ID NO:2 provides passage 1 of PRRSV isolate 96V198.

SEQ ID NO:3 provides passage 9 of PRRSV isolate 96V198.

SEQ ID NO:4 provides passage 14 of PRRSV isolate 96V198.

SEQ ID NO:5 provides passage 49 of PRRSV isolate 96V198.

SED ID NOS: 6-8 provide PCR primer sequences.

300

DETAILED DESCRIPTION OF THE INVENTION

As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

305

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

310

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, *et al.* Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Maniatis *et al.* Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984).

315

320

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

325

330 virus strains generally evidence about 80-95% nucleotide sequence identity with North American strains (see the default values for the Clustal W algorithm in the Lasergene software suite, DNASTAR, Inc.), and North American strains are similarly about 85% to 100% identical to each other.

"European PRRS virus" (PRRSV-1, or formerly PRRSV Type 1) refers to any
335 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, the Lelystad virus, see also U.S. Patents 5,620,691 and 6,197,310). European PRRS virus also generally refers to viruses having full length nucleotide sequences that are about 80% identical or higher to the Lelystad isolate. It should be noted that within PRRSV-
340 1, subtypes have come to be identified, so that Lelystad virus has been denominated the prototype for subtype 1, and the Lena virus (U. Karniychuk et al., BMC Veterinary Research, 2010, Vol 6, No. 30) has become denominated as the prototype of subtype 3. These two viruses approximately span the maximum current divergence of PRRSV-1, and thus, for example, using ORF5, Lelystad and Lena are about 82.8% identical at the nucleotide level,
345 and using full genomes, Lelystad and Lena are about 80.7% identical at the nucleotide level. Optimization of alignments varies slightly based on algorithm parameters, but the reader is generally referred to the default values for the Clustal W algorithm in the Lasergene software suite (DNASTAR, Inc.).

European PRRS strains are generally only about 60% identical to both North
350 American and Asian (Chinese) strains. For example, using ORF5 nucleotide sequence (which tends to give a good to fair estimate of entire genome when entire genomes are not known), the PRRSV-1 prototype Lelystad Virus and the North American prototype VR2332 strain are about 65.0% identical at the nucleotide level. Using full genome nucleotide sequence, the prototype Lelystad Virus and the prototype VR2332 virus are about 58.6%
355 identical at the nucleotide level, again using the default values for the cited Clustal W algorithm.

"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
360 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
365 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.

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

"Host cell capable of supporting PRRS virus replication" means a cell which is
375 capable of generating infectious PRRS when infected with a virus of the invention. Such
cells include porcine cells of the monocyte/macrophage lineage such as porcine alveolar
macrophage cells and derivatives, MA-104 monkey kidney cells and derivatives such as
MARC-145 cells; and cells transfected with a gene encoding a receptor for the PRRS virus
(see U.S. Patent 9,102,912 referring to assignment of the mammalian CD163 surface
380 protein as the normal PRRS virus cell receptor). The term "host cell capable of supporting
PRRS virus replication" may also include cells within a live pig.

"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.

385 "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. The vaccines and method
protocols of the invention are applicable to all swine, whether male of any age, or female of
any age to include sows and gilts, and such methods also are capable of protecting male
and female piglets indirectly via protection administered to the mother sow. The term "PRRS
390 virus", as used herein, unless otherwise indicated, means any strain of either the North
American, Asian or European PRRS viruses.

"PRRS" encompasses disease symptoms in swine caused by a PRRS virus
(PRRSV) infection. Examples of such symptoms include, but are not limited to, fever,
abortion in pregnant females, respiratory distress, lung lesions, loss of appetite, and
395 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.

"Transfected host cell" means practically any host cell which, when transfected with PRRS virus RNA, can produce at least a first round of PRRS virions.

400 An "infectious DNA molecule", for purposes of the present invention, is a DNA molecule that encodes the necessary elements to support replication, transcription, and translation into a functional virion from a suitable host cell.

Likewise, an "isolated polynucleotide molecule" refers to a composition of matter comprising a polynucleotide molecule of the present invention purified or controlled to any
405 detectable degree from its naturally occurring state, if any.

For purposes of the present invention, the nucleotide sequence of a second polynucleotide molecule (either RNA or DNA) is "homologous" to the nucleotide sequence of a first polynucleotide molecule, or has "identity" to said first polynucleotide molecule, where
410 the nucleotide sequence of the second polynucleotide molecule encodes the same polyaminoacid as the nucleotide sequence of the first polynucleotide molecule as based on the degeneracy of the genetic code, or when it encodes a polyaminoacid that is sufficiently similar to the polyaminoacid encoded by the nucleotide sequence of the first polynucleotide molecule so as to be useful in practicing the present invention. Homologous polynucleotide
415 sequences also refers to sense and anti-sense strands, and in all cases to the complement of any such strands. For purposes of the present invention, a polynucleotide molecule is useful in practicing the present invention, and is therefore homologous or has identity, where it can be used as a diagnostic probe to detect the presence of PRRS virus or viral polynucleotide in a fluid or tissue sample of an infected pig, e.g. by standard hybridization or
420 amplification techniques. Generally, the nucleotide sequence of a second polynucleotide molecule is homologous to the nucleotide sequence of a first polynucleotide molecule if it has at least about 70% nucleotide sequence identity to the nucleotide sequence of the first polynucleotide molecule as based on the BLASTN algorithm (National Center for Biotechnology Information, otherwise known as NCBI, (Bethesda, Maryland, USA) of the
425 United States National Institute of Health). In a specific example for calculations according to the practice of the present invention, reference is made to BLASTP 2.2.6 [Tatusova TA and TL Madden, "BLAST 2 sequences- a new tool for comparing protein and nucleotide sequences." (1999) FEMS Microbiol Lett. 174:247-250.]. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap

430 extension penalty of 0.1, and the "blosum62" scoring matrix of Henikoff and Henikoff (Proc. Nat. Acad. Sci. USA 89:10915-10919. 1992). The percent identity is then calculated as: Total number of identical matches x 100/ divided by the length of the longer sequence+number of gaps introduced into the longer sequence to align the two sequences.

Preferably, a homologous nucleotide sequence has at least about 75% nucleotide
435 sequence identity, even more preferably at least about 80%, 85%, 90% 95%, 96%, 97%, 98% and 99% nucleotide sequence identity. Since the genetic code is degenerate, a homologous nucleotide sequence can include any number of "silent" base changes, i.e. nucleotide substitutions that nonetheless encode the same amino acid.

A homologous nucleotide sequence can further contain non-silent mutations, i.e.
440 base substitutions, deletions, or additions resulting in amino acid differences in the encoded polyaminoacid, so long as the sequence remains at least about 70% identical to the polyaminoacid encoded by the first nucleotide sequence or otherwise is useful for practicing the present invention.

In this regard, certain conservative amino acid substitutions may be made which
445 are generally recognized not to inactivate overall protein function: such as in regard of positively charged amino acids (and vice versa), lysine, arginine and histidine; in regard of negatively charged amino acids (and vice versa), aspartic acid and glutamic acid; and in regard of certain groups of neutrally charged amino acids (and in all cases, also vice versa), (1) alanine and serine, (2) asparagine, glutamine, and histidine, (3) cysteine and serine, (4)
450 glycine and proline, (5) isoleucine, leucine and valine, (6) methionine, leucine and isoleucine, (7) phenylalanine, methionine, leucine, and tyrosine, (8) serine and threonine, (9) tryptophan and tyrosine, (10) and for example tyrosine, tryptophan and phenylalanine. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art
455 as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions may be found in WO 97/09433, page 10, published Mar. 13. 1997 (PCT/GB96/02197, filed Sep. 6, 1996. Alternatively, conservative amino acids can be grouped as described in Lehninger, (Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp. 71-77). Additional suitable conservative changes and
460 the application thereof are described below.

Homologous nucleotide sequences can be determined by comparison of nucleotide sequences, for example by using BLASTN, above. Alternatively, homologous nucleotide

sequences can be determined by hybridization under selected conditions. For example, the nucleotide sequence of a second polynucleotide molecule is homologous to SEQ ID NO:5 (or any other particular polynucleotide sequence) if it hybridizes to the complement of SEQ ID NO:5 under moderately stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.2xSSC/0.1% SDS at 42°C (see Ausubel et al editors, *Protocols in Molecular Biology*, Wiley and Sons, 1994, pp. 6.0.3 to 6.4.10), or conditions which will otherwise result in hybridization of sequences that encode a PRRS virus as defined below. Modifications in hybridization conditions can be empirically determined or precisely calculated based on the length and percentage of guanosine/cytosine (GC) base pairing of the probe. The hybridization conditions can be calculated as described in Sambrook, *et al.*, (Eds.), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York (1989), pp. 9.47 to 9.51.

In another embodiment, a second nucleotide sequence is homologous to SEQ ID NO:5 (or any other sequence of the invention) if it hybridizes to the complement of SEQ ID NO:5 under highly stringent conditions, e.g. hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% SDS, 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C, as is known in the art (Ausubel et al. *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York, 1989).

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.

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." The mutations may be carried out by standard methods known in the art, e.g. site directed mutagenesis (see e.g. Sambrook et al.(1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) of an infectious copy as described (e.g. Meulenberg et al., *Adv. Exp. Med. Biol.*, 1998, 440:199-206).

In connection with providing preferred European PRRS virus sequences that are both safe and effective when administered to pre-weaning piglets, the following are among the amino acid positions that may be modified. In identifying these specific mutations, it is

of course noted that conservative substitutions for these preferred amino acid residues, as defined immediately above, may also be used. In identifying the preferred encoded amino acids, the corresponding/original amino acids of the wild isolate of strain 96V198 are also shown by parenthesis, thus passage 0 (SEQ ID NO:1) is compared to passage 49 (the preferred vaccine material, SEQ ID NO:5). Thus, for the amino acid sequence encoded from ORF1a, S at amino acid position 19 (N); Y at amino acid position 24 (F); A at amino acid position 156 (T); Y at amino acid position 157 (H); D at amino acid position 268 (N); H at amino acid position 294 (Y); Y at amino acid position 416 (C); S at amino acid position 742 (P); L at amino acid position 884 (F); P at amino acid position 908 (S); K at amino acid position 916 (E); K at amino acid position 977 (E); S at amino acid position 1138 (P); F at amino acid position 1160 (L); S at amino acid position 1500 (P); R at amino acid position 2094 (Q); P at amino acid position 2254 (S); and L at amino acid position 2290 (F). For the amino acid sequence encoded from ORF1b, S at amino acid position 567 (N); and H at amino acid position 912 (Q). For the amino acid sequence encoded from ORF2a, L at amino acid position 22 (S); F at amino acid position 88 (V); M at amino acid position 94 (I); and F at amino acid position 95 (L). For the amino acid sequence encoded from ORF2b, L at amino acid position 47 (F). For the amino acid sequence encoded from ORF3, S at amino acid position 52 (T). For the amino acid sequence encoded from ORF4, T at amino acid position 151 (I). For the amino acid sequence encoded from ORF5, F at amino acid position 20 (L); and D at amino acid position 37 (N). For the amino acid sequence encoded from ORF5a, V at amino acid position 18 (A); and R at amino acid position 35 (Q).

Accordingly, the present invention further provides a method for making a genetically modified European PRRS virus, which method comprises mutating the DNA sequence encoding an infectious RNA molecule which encodes the PRRS virus as described above, and expressing the genetically modified PRRS virus using a suitable expression system. A genetically modified PRRS virus 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.

The North American PRRSV N protein sequences are highly conserved and the reported sequences have about 93-100% identity with each other. The North American and European PRRSV N proteins are about 57-59% identical and share common structural motifs. Generally, when comparing PRRS encoding sequences and isolates, which might

be numbered differently as to specific nucleotides or encoded amino acids, identification of the proper regions are readily achieved by identifying preserved characteristic amino acids
535 in a PRRS strain of interest and aligning it with a reference strain.

Recombinant DNA technology comprises extremely varied and powerful molecular biology techniques aimed at modifying nucleic acids at the DNA level and makes it possible to analyze and modify genomes at the molecular level. In this respect, viruses such as the PRRS virus because of the modest size of its genome is particularly amenable
540 to such manipulations. However, recombinant DNA technology is not immediately applicable to non-retroviral RNA viruses because these viruses do not encompass a DNA intermediate step in their replication. For such viruses, infectious cDNA clones have to be developed before recombinant DNA technology can be applied to their genome to generate modified virus. Infectious clones can be derived through the construction of full-length (genomic
545 length) cDNA (here used in the broad sense of a DNA copy of RNA and not only in the strict sense of a DNA copy of mRNA) of the virus under study, after which an infectious transcript is synthesized *in vivo* in cells transfected with the full-length cDNA, but infectious transcripts can also be obtained by *in vitro* transcription from full-length cDNA in a plasmid having a prokaryotic promoter in the presence of a transcription cocktail, or again *in vitro* using
550 ligated partial-length cDNA fragments that comprise the full viral genome. In all cases, the transcribed RNA carries all the modifications that have been introduced to the cDNA and can be used to further passage the thus modified virus.

The preparation of an infectious clone of a European PRRS virus isolate or Lelystad virus is described in U.S. Pat. No. 6,268,199 which is hereby fully incorporated by
555 reference. The preparation of an infectious cDNA clone of a North American PRRS virus isolate designated P129 (Lee *et al.*, 2005; Yoo *et al.*, 2004) is described in U.S. Pat. No. 6,500,662 which is hereby incorporated fully by reference. The sequence of P129 cDNA is disclosed in Genbank Accession Number AF494042 and in U.S. Pat. No. 6,500,662. Our work below makes use of such an infectious clone which in the context of a plasmid is
560 expressed by the CMV immediate early promoter and has been designated pCMV-S-P129 and is also disclosed within U.S. Pat. No. 6,500,662. As described in U.S. Pat. No. 6,500,662 there are other plasmids and promoters suitable for use here.

Given the complete sequence of any open reading frame of interest and the location of an amino acid residue of interest, one of ordinary skill need merely consult a
565 codon table to design changes at the particular position desired.

Codons constitute triplet sequences of nucleotides in mRNA and their corresponding cDNA molecules. Codons are characterized by the base uracil (U) when present in a mRNA molecule but are characterized by base thymidine (T) when present in DNA. A simple change in a codon for the same amino acid residue within a polynucleotide
570 will not change the sequence or structure of the encoded polypeptide. It is apparent that when a phrase stating that a particular 3 nucleotide sequence "encode(s)" any particular amino acid, the ordinarily skilled artisan would recognize that the table above provides a means of identifying the particular nucleotides at issue. By way of example, if a particular three nucleotide sequence encodes lysine, the table above discloses that the two possible
575 triplet sequences are AAA and AAG. Glycine is encoded by GGA, GGC, GGT (GGU if in RNA) and GGG. To change a lysine to glycine residue in an encoded protein one might replace a AAA or AAG triplet with any of by GGA and GGC, GGT or GGG in the encoding nucleic acid.

Studies on the immunobiology of PRRS virus are suggestive that the interaction
580 of PRRS virus with PDCs merits examination. This cell type represents 0.2%-0.8% of peripheral blood mononuclear cells in humans, mice, rats, pigs and monkeys. Despite its scarcity, this cell is an important component of the innate immune system and is capable of secreting copious amounts of IFN- α following viral stimulation. It is through the secretion of IFN- α that PDCs play a major role in regulating antiviral innate and adaptive immunity since
585 they promote the function of natural killer cells, B cells, and T cells. Furthermore, the maturation of porcine monocyte derived dendritic cells (MoDC) is aided by the IFN- α secreted by PDCs resulting in an enhanced ability of MoDCs to present antigen and activate T cells. At a later stage of viral infection, PDCs differentiate into a unique type of mature dendritic cell, which directly regulates the function of T cells and direct the differentiation of
590 T cells into cells capable of secreting IFN- γ , which is a major mediator of antiviral immunity against viruses including PRRS virus. Not surprisingly there are human viruses, such as respiratory syncytial virus and measles virus, which are known to suppress the ability of PDCs to secrete IFN- α . This inhibitory effect is thought to play a role in the predominance of a humoral immune response and the associated immunopathology observed as a result
595 of the infection with these viruses, as well as in the increased susceptibility of the host to secondary bacterial and viral infections.

As aforementioned, there are numerous known strains and isolates of European, North American and Chinese PRRS, and novel strains continue to evolve or to be isolated. Although a high level of amino acid sequence homology exists between all these strains,

600 those skilled in the art will immediately recognize that some variation does exist, and indeed advantage can be taken of these differences and similarities to further improve the phenotypic properties of all vaccine strains.

First, in regard of all of the amino acid motifs defined by SEQ ID NOS as specified
605 below, it is generally possible to inspect the corresponding expressed protein sequences from any other European PRRS, to find the corresponding amino acid motif, even if additional changes have occurred in such other strains, as a result of evolution, causing substitutions and/or deletions or additions. Thus, it should be readily possible to identify the comparable motif in another PRRS strain if, for example, the valine therein is replaced by
610 isoleucine or leucine, or any other residue, or if a residue is simply missing or an additional residue added. Numerous computer programs exist to identify alignments and thus determine if polypeptide sequence motifs correspond, for example the so-called Blosum tables (based on a given level of percent identity), see S. Henikoff et al. "Amino Acid Substitution matrices from protein blocks", Proc Natl Acad Sci, USA, 89(22), pp. 10915-
615 10919, Nov 15, 1992., and see also A. L. Lehninger et al. Principles of Biochemistry, 2005, MacMillan and Company, 4th edition. Conservative amino acid changes are also recognized based on categorization into 5 overall groups: sulfydryl (Cys); aromatic (Phe, Tyr, and Trp); basic (Lys, Arg, His); aliphatic (Val, Ileu, Leu, Met), and hydrophilic (Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser and Thr). Thus it is within the practice of the invention to modify any
620 European PRRS encoding nucleotide sequence to incorporate at the appropriate and corresponding position, any of the amino acid changes provided by SEQ ID NO:5 even if one or more of the other amino acids adjacent to the designated position have been added, deleted or substituted. Such amino acid changes may, of course, be introduced into the corresponding encoding nucleotide sequences of the virus by site directed mutagenesis,
625 PCR, and other techniques as are well known in the art.

General measure of attenuation

To demonstrate that a particular genetically modified strain is attenuated an experiment described as follows may be used.

630 At least 10 gilts per group are included in each trial, which are derived from a PRRSV-free farm. Animals are tested free of PRRS virus specific serum antibodies and negative for PRRSV. All animals included in the trial are of the same source and breed. The allocation of the animals to the groups is randomized.

Challenge is performed at day 90 of pregnancy with intranasal application of 1 ml
635 PRRSV with 10^5 TCID₅₀ per nostril. There are at least three groups for each test setup: One
group for wild type virus; one test group for challenge with the possibly attenuated virus; and
one strict control group.

The study is deemed valid when the strict controls stay PRRSV-negative over the
time course of the study and at least 25% less live healthy piglets are born in the wild type
640 challenged group compared to the strict controls.

Attenuation, in other words less virulence, is defined as the statistical significant
change of one or more parameters determining reproductive performance or other
symptomology:

Significant reduction in at least one of the following parameters for the test group (possibly
645 attenuated virus) compared to the unmodified parental strain infected group would be an
indication of attenuation:

- a) frequency of stillborns
- b) abortion at or before day 112 of pregnancy
- c) number of mummified piglets
- 650 d) number of less lively and weak piglets
- e) pre-weaning mortality.

Furthermore a significant increase in one of the following parameters for the test
group compared the unmodified parental strain infected group is preferred:

- f) number of piglets weaned per sow
- 655 g) number of live healthy piglets born per sow.

In the alternative, respiratory symptoms and other symptoms of PRRSV infection could be
examined to establish attenuation.

An attenuated strain is valuable for the formulation of vaccines. The present
vaccine is effective if it protects a pig against infection by a PRRS virus. A vaccine protects
660 a pig against infection by a PRRS virus if, after administration of the vaccine to one or more
unaffected pigs, a subsequent challenge with a biologically pure virus isolate (e.g., any
European wild type) results in a lessened severity of any gross or histopathological
changes (e.g., lesions in the lung) and/or of symptoms of the disease, as compared to those
changes or symptoms typically caused by the isolate in similar pigs which are unprotected
665 (i.e., relative to an appropriate control). More particularly, the present vaccine may be shown

to be effective by administering the vaccine to one or more suitable pigs in need thereof, then after an appropriate length of time (e.g., 4 weeks), challenging with a large sample ($10^{(3-7)}$ TCID₍₅₀₎) of a biologically pure PRRSV isolate. A blood sample is then drawn from the challenged pig after about one week, and an attempt to isolate the virus from the blood sample is then performed. Isolation of a large amount of the virus is an indication that the vaccine may not be effective, while isolation of reduced amounts of the virus (or no virus) is an indication that the vaccine may be effective.

Thus, the effectiveness of the present vaccine may be evaluated quantitatively (i.e., a decrease in the percentage of consolidated lung tissue as compared to an appropriate control group) or qualitatively (e.g., isolation of PRRSV from blood, detection of PRRSV antigen in a lung, tonsil or lymph node tissue sample by an immunoassay). The symptoms of the porcine reproductive and respiratory disease may be evaluated quantitatively (e.g., temperature/fever) or semi-quantitatively (e.g., the presence or absence of one or more symptoms or a reduction in severity of one or more symptoms, such as cyanosis, pneumonia, lung lesions etc.).

An unaffected pig is a pig which has either not been exposed to a porcine reproductive and respiratory disease infectious agent, or which has been exposed to a porcine reproductive and respiratory disease infectious agent but is not showing symptoms of the disease. An affected pig is one which shows symptoms of PRRS or from which PRRSV can be isolated.

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, which is incorporated herein by reference.

Vaccines of the present invention can further comprise one or more additional immunomodulatory components such as, e.g., an adjuvant or cytokine, among others. Non-limiting examples of adjuvants that can be used in the vaccine of the present invention

include the RIBI adjuvant system (Ribi Inc., Hamilton, Mont.), alum, mineral gels such as
700 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 Mass.), SAF-M (Chiron, Emeryville Calif.),
AMPHIGEN.RTM. adjuvant, saponin, Quil A or other saponin fraction, monophosphoryl lipid
A, and Avridine lipid-amine adjuvant. Non-limiting examples of oil-in-water emulsions useful
705 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.RTM. 85 detergent (ICI Surfactants), 0.7% (v/v) TWEEN.RTM. 80 detergent (ICI
Surfactants), 2.5% (v/v) ethanol, 200 pg/ml Quil A, 100 [mgr]g/ml cholesterol, and 0.5% (v/v)
lecithin. Modified SEAM 1/2 is an oil-in-water emulsion comprising 5% (v/v) squalene, 1%
710 (v/v) SPAN.RTM. 85 detergent, 0.7% (v/v) Tween 80 detergent, 2.5% (v/v) ethanol, 100
.mu.g/ml Quil A, and 50 .mu.g/ml cholesterol. Other immunomodulatory agents that can be
included in the vaccine include, e.g., one or more interleukins, interferons, or other known
cytokines.

Vaccines of the present invention can optionally be formulated for sustained
715 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
720 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*
725 *Pharmaceutical Sciences*, Vol. 45, M. Dekker, N.Y., which is also incorporated herein by
reference. 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. Pat. No.
3,137,631; U.S. Pat. No. 3,959,457; U.S. Pat. No. 4,205,060; U.S. Pat. No. 4,606,940; U.S.
730 Pat. No. 4,744,933; U.S. Pat. No. 5,132,117; and International Patent Publication WO
95/28227, all of which are incorporated herein by reference.

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. Pat. No. 4,016,100; U.S. Pat. No. 4,452,747; U.S. Pat. No.
735 4,921,706; U.S. Pat. No. 4,927,637; U.S. Pat. No. 4,944,948; U.S. Pat. No. 5,008,050; and
U.S. Pat. No. 5,009,956, all of which are incorporated herein by reference.

An effective amount of any of the above-described vaccines can be determined by
conventional means, starting with a low dose of virus, viral protein plasmid or viral vector,
740 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
745 after consideration of the results from other animal studies (see Examples 3-7 below).

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
750 by a doctor or veterinarian based on analysis of all relevant factors, some of which are
described above.

The effective dose amount of virus, protein, infectious DNA molecule, plasmid, or
viral vector, of the present invention can be determined using known techniques, taking into
755 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
760 vaccine of the present invention preferably ranges from about 0.1mg to about 100mg, more
preferably from about 1mg to about 10mg, even more preferably from about 10mg to about
1mg. The dose amount of an infectious DNA molecule of the present invention in a vaccine
of the present invention preferably ranges from about 0.1mg to about 100mg, more
preferably from about 1mg to about 10mg, even more preferably from about 10mg to about
765 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.

770 Suitable doses for viral protein or peptide vaccines according to the practice of the present invention range generally from 1 to 50 micrograms per dose, or higher amounts as may be determined by standard methods, with the amount of adjuvant to be determined by recognized methods in regard of each such substance. In a preferred example of the invention relating to vaccination of swine, an optimum age target for the animals is between
775 about 1 and 21 days, which at pre-weaning, may also correspond with other scheduled vaccinations such as against *Mycoplasma hyopneumoniae* or PCV. Additionally, a preferred schedule of vaccination for breeding sows would include similar doses, with an annual revaccination schedule.

780 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.

785 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. By way of example, vaccines may be delivered orally, parenterally, intradermally, subcutaneously, intramuscularly, intranasally or intravenously.
790 Oral delivery may encompass, for example, adding the compositions to the feed or drink of the animals. Factors bearing on the vaccine dosage include, for example, the weight and age of the pig.

 The present invention further provides a method of preparing a vaccine comprising a PRRS virus, infectious RNA molecule, plasmid, or viral vector described
795 herein, which method comprises combining an effective amount of one of the PRRS virus, infectious RNA molecule, plasmid, or viral vector of the present invention, with a carrier acceptable for pharmaceutical or veterinary use.

 In addition the live attenuated vaccine of the present invention can be modified as described in U.S. Pat. No. 6,500,662 to encode a heterologous antigenic epitope which is
800 inserted into the PRRS viral genome using known recombinant techniques. See also U.S. Patent 7,132,106 which is incorporated by reference in its entirety. Antigenic epitopes useful as heterologous antigenic epitopes for the present invention include antigenic epitopes from a swine pathogen other than PRRS virus which include, but are not limited to, an antigenic

805 epitope from a swine pathogen selected from the group consisting of 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, torque teno virus, *Actinobacillus pleuropneumoniae*, *Actinobacillus suis*, *Bacillus anthracis*, *Bordetella bronchiseptica*, *Clostridium haemolyticum*, *Clostridium perfringens*, *Clostridium tetani*,
810 *Escherichia coli*, *Erysipelothrix rhusiopathiae*, *Haemophilus parasuis*, *Leptospira* spp., *Mycoplasma hyopneumoniae*, *Mycoplasma hyorhinis*, *Mycoplasma hyosynoviae*, *Pasteurella multocida*, *Salmonella choleraesuis*, *Salmonella typhimurium*, *Streptococcus equismilis*, 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
815 databases on the worldwide web, such as at Genbank from the (USA) National Center for Biotechnology Information.

Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention
820 described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are
825 intended as aspects of the invention. It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Accordingly, a PRRS vaccine based on the SEQ ID NO:5 virus will be seen as representative of those having a safety and efficacy profile that provides the dramatic improvement of permitting successful vaccination of piglets, as early even as the first day of
830 life, with long lasting immunity out to about 6 months of age. Such unprecedented improvements in vaccine performance are surprisingly provided by culturing and attenuating the virus in a cell culture environment that maximizes relevance to swine biology, including by permitting appropriate and safe replication of the virus in a swine host while not downregulating the needed host immune response. Such benefits are provided to piglets,
835 boars, and sows and gilts (both before and during pregnancy); and in all cases whether the animal is seropositive or seronegative at the time of vaccination.

It is also readily apparent that the culturing improvements of the present invention are equally applicable to European PRRS viruses irrespective of whether they are, were, or come to be manipulated by recombinant means, or have only been isolated, maintained, or
840 cultured and adapted by primarily non-recombinant means; in all cases including whether any culturing cells used are, or are not themselves recombinant.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

The following examples are intended to illustrate but not limit the invention.

845 Example 1--Generation of the BHK21-C12-26 cell line

The generation of the BHK21-C12 cell line, the parent of the BHK21-C12-26 cell line used in these studies, was previously described in US 9,102,912. In this patent, the BHK21-C12 cell line is referred to as BHK/CMV/v1 #12 in Example 14 and Figure 6. Briefly, the commonly used baby hamster kidney cell line BHK-21 was transfected with the plasmid
850 pCMV-susCD163v1, which contains a truncated variant (v1) of the porcine (sus) CD163 PRRSV receptor gene, under control of the CMV promoter. The plasmid also contains a kanamycin/neomycin resistance gene under the control of dual prokaryotic/eukaryotic promoters for selection in E. coli and mammalian cells, respectively. Following transfection, the cells were subjected to simultaneous selection with G418 (Geneticin, a neomycin
855 analog) and single cell cloning in 96-well plates. One of the resulting clones (#12, or "C12") was chosen for further use and designated BHK21-C12.

The BHK21-C12 cell line was serially passaged 65 times under G418 selection and retained the PRRSV-permissive phenotype. By passage 64 it was observed that a few cells survived infection with the PRRS virus, indicating the early stages of phenotypic instability.
860 Additional single cell cloning was performed, and many new clones were evaluated for the PRRSV-permissive phenotype and for general growth characteristics. Of these, clone #26 was selected for being superior to other clones and to the parental BHK21-C12 cell line. Clone #26 was designated BHK21-C12-26, and was used in the attenuation of PRRS virus 96V198.

865 Example 2- additional cells appropriate for adaptation and culturing of virus that is safe and effective at very early age, such as for vaccination at Day 1 after birth

PK-9 cells are a transgenic cell line derived by stably transfecting the PK0809 porcine kidney cell line with a plasmid encoding a deleted version of the porcine CD163 gene and the neomycin resistance gene. Details of the construction and characterization of the PK-9 cell line have been described previously (see U.S. Patent 9,102,912).

It should be noted that adaptation of a first passage virus from PAM cells to growth on PK-9 cells may be difficult, and required several attempts with multiple parallel lineages. In this case, infection can be monitored by immunofluorescence of duplicate wells using FITC-conjugated monoclonal antibody SDOW17 specific for the viral nucleocapsid protein (Rural Technologies Inc, Brookings South Dakota). Early passages may result in a few small foci, and not generate enough cell-free virus particles to initiate infection of a fresh monolayer. These passages can nonetheless be accomplished by treating the infected monolayer with Accutase (a trypsin substitute) and reseeding the cells in multiple wells with fresh medium, with or without the addition of non-infected PK-9 cells. After several such passages, some lineages should show a clear increase in the frequency and size of fluorescent foci. Some of these should acquire the ability to be passaged using cell-free virus fluids. Generally, it is expected that appropriate passages evidence a reduced ability to inhibit alpha interferon.

The safety and efficacy of the European PRRS vaccines of the invention are further evidenced by the experimental protocols and data comprising Examples 3-7, which follow directly.

Example 3 - Duration of immunity provided by the European PRRS MLV administered to 1-day-old piglets with subsequent challenge with a further European PRRSV isolate at approximately 26 weeks post-vaccination.

The objective of the study was to evaluate the duration of immunity (DOI) of the European PRRS modified live vaccine (the virus encoded by SEQ ID NO:5) in pigs vaccinated at 1 day of age by the intramuscular (IM, group T02) or the intranasal (IN, group T03) route, followed by a respiratory challenge at 26 weeks post-vaccination with a different European PRRSV isolate. The primary variable in determining the efficacy was viral load in serum (viremia) of treatment groups T02 and T03 in comparison to T01 pigs. Lung lesions, rectal temperatures, shedding, clinical signs and body weight were compared as secondary variables.

At 1 day of age, pigs from groups T02 and T03 were administered with a single 2 mL dose of IVP via the intramuscular (T02) or the intranasal (T03) route. Pigs from the control group (T01) received 2 ml intramuscular and 2 ml intranasal of Saline Solution. At 26 weeks post-vaccination (182 days), pigs were challenged intranasally with the EU PRRSV isolate Olot/91 (see J. Duran et al., Virus Genes, vol 14 No. 1., 1997, pp 19-29 and Z. Lu et al., Virology Journal, vol 11, No 42, 2014) as a respiratory challenge. During the challenge phase, blood samples, nasal and oral swabs, clinical observations and rectal temperatures were collected every 3-4 days until day of necropsy. Pigs were weighed before vaccination, before challenge, and at necropsy. Nine or ten days post-challenge, pigs were euthanized and necropsied. Upon necropsy, lungs were evaluated for the presence of PRRSV lesions and scored. The test was valid since all T01 pigs remained PRRSV viro-negative throughout the vaccination period and no confounding disease factors were detected.

A protective effect of both IM and IN vaccination was observed when comparing viral load in serum between groups. Both groups vaccinated with the IVP (T02 and T03) had significantly ($P \leq 0.05$) lower viral titers compared to the control group at all sampling days post-challenge (3, 6, 8 and 9/10, corresponding to study days 185, 188, 190 and 191/192). Efficacy was also supported by the significant reduction of PRRSV-associated lung lesions at necropsy, as well as the significant reduction in nasal and oral shedding in both vaccinated groups compared to the control group.

Comparison between vaccinated groups revealed that at 3 days post-challenge, the group vaccinated by the IN route (T03) had significantly lower viremia, nasal shedding and oral shedding compared to the group vaccinated by the IM route (T02). These results indicate that, under the conditions of the present study, the protection obtained following the IN vaccination was stronger than following IM vaccination.

Vaccination induced the development of PRRSV-specific antibodies within 28 days post-vaccination. All vaccinated pigs were seropositive to PRRS antibodies at challenge (26 weeks post-vaccination), indicating that both administration routes were able to elicit a strong and protective antibody response to vaccination. However, the level of antibody titres detected at challenge were significantly higher in the group vaccinated by the IN route compared to the group vaccinated intramuscularly, fact that would explain the significant reduction in viral load detected in both serum and excretion routes in the IN group compared to the IM group at 3 days post-challenge.

In conclusion, the results from the present study demonstrated that a single administration of the EU PRRSV MLV vaccine (SEQ ID NO:5) containing a dose of 2.5 log₁₀ CCID₅₀ to 1 day-old pigs by the intramuscular or the intranasal route conferred a protective duration of immunity of 6.5 months (26 weeks).

Abbreviations used in Examples 3-7 include: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; AE, Adverse Event; CCID₅₀, Cell Culture Infectious Dose 50%; CP, Control Product; DC, Day of Challenge; DCF, Data Capture Form; DRAC, Daily Review Animal Care; ID, identification; IF, Immunofluorescence; IM, Intramuscular; IN, Intranasal; IV, Intravenous; IVP, Investigational Veterinary Product; MDA, Maternally Derived Antibodies; MLV, Modified Live Vaccine; MSF, Master Study File; MSV, Master Seed Virus; NA, Not Applicable; PAM, Porcine Alveolar Macrophages; PRRSV, Porcine Reproductive and Respiratory Syndrome Virus; PBS, Phosphate Buffered Saline; RT-qPCR, Quantitative Reverse-Transcription Polymerase Chain Reaction; SART, Sick Animal Report and Treatment; SOP, Standard Operating Procedure; S/P, Sample to positive; and TBD, To Be Determined.

Design

Group	Treatment Description	Dosage (Actual titre)	Route	Day of Admin	Day of Challenge (DC)	Challenge dose (Actual titre)	End of Study (DN)	N
T01	CP	2 mL + 2 mL	IM + IN	D0	D182	5.7 log ₁₀ CCID ₅₀ /pig	D191/ D192	20
T02	IVP	2.2 log ₁₀ CCID ₅₀ /2.0 mL	IM	D0	D182		D191/ D192	18
T03	IVP	2.2 log ₁₀ CCID ₅₀ /2.0 mL	IN	D0	D182		D191/ D192	17

At 1 day of age, a 2 mL dose (IM route) and a 2 mL dose (IN route) of the CP was administered to T01 piglets. Also, a single 2.0 mL dose of the IVP was administered to T02 piglets by IM route and to T03 piglets by IN route. Twenty-six weeks post-vaccination, pigs were challenged with PRRSV Olot/91 and at 9-10 post-challenge, they were euthanized and necropsied. Day 0 = Day of vaccination.

965 Randomisation

Immediately after birth, piglets were cross-fostered such that piglets were randomized and spread as even as possible over all sows. At weaning, sows were removed and piglets were housed in pens in the source farm (1 pen per treatment).

970 Certain parameters for the animals are as follows:

Species/breed:	Porcine crossbred
Initial age on day 0:	1 day (24±12 h) old
Initial weight on day 0	NA
Sex:	Male and female
Origin:	Farm Cal Marquès Zoetis Manufacturing & Research Spain S.L. Ctra Camprodon s/n Finca "La Riba" 17813 Vall de Bianya (Girona), Spain
Serological status:	Seronegative to PRRSV
Reproductive status:	NA
Identification method:	Ear tags
Ownership:	Zoetis Manufacturing & Research Spain S.L.

Twelve pregnant sows were used to get a total of 117 piglets. The day before the expected farrowing date, parturition was induced with an intramuscular injection of cloprostenol (Cyclix® Porcino, Virbac). All sows farrowed the day after (D-1). To reduce the duration of parturition and the number of stillborn piglets, all sows but two (71 and 78 that already finished farrowing), were injected with oxytocin (Partovet® DFV).

During the vaccination phase, 8 piglets died or had to be euthanized. From the remaining 109 piglets, 55 were for the present study.

980

To reduce aggressive and sexual behaviour in male pigs at older age, piglets were castrated at 6 days of age following site-specific animal welfare procedures. Three piglets (232, 284 and 286) could not be castrated because they had scrotal hernia.

985

Around 1 month post-vaccination, two sudden deaths with identical pathological findings together with the presence of white spots on the floor and some pigs showing white urine at the end of the micturition process suggested a bacterial infection affecting the urinary tract. This process was not considered to have an impact on the study results since all animals responded well to treatment and no other case appeared during the rest of the vaccination phase (next 3 months until challenge). •Any animal with an injury or clinical illness unrelated to the test procedure that may affect the health of the animal and evaluation of the results may be withdrawn from the study at the discretion of the investigator. The Investigational Veterinary Product (IVP) was provided from a freeze dried fraction that was resuspended in saline solution at $2.5 \log_{10} \text{CCID}_{50}/\text{ML}$ (SEQ ID NO: 5 virus)

990

995

Reconstitution

At day 0, the IVP was diluted with vaccine diluent (lot T22019) to match the target titer ($2.5 \log_{10} \text{CCID}_{50}/2 \text{ mL}$). One aliquot of the IVP was collected for titration on BHK-21-C12-26 cells to confirm the dosage. An additional IVP sample was frozen ($-80 \pm 10^{\circ}\text{C}$) and stored as a retention sample. Titration on BHK-21-C12-26 cells was performed following local standard procedures. The reconstituted and diluted vaccine had a titer of $10^{1.9} \text{CCID}_{50}/\text{ml}$, which corresponds to $10^{2.2} \text{CCID}_{50}/2 \text{ mL}$ ($2.2 \log_{10} \text{CCID}_{50} / 2 \text{ ml}$). At day 0, the IVP was diluted with vaccine diluent (lot T22019) to match the target titer ($2.5 \log_{10} \text{CCID}_{50}/2 \text{ mL}$). One aliquot of the IVP was collected for titration on BHK-21-C12-26 cells to confirm the dosage. An additional IVP sample was frozen ($-80 \pm 10^{\circ}\text{C}$) and stored as a retention sample. Titration on BHK-21-C12-26 cells was performed following local standard procedures. The reconstituted and diluted vaccine had a titer of $10^{1.9} \text{CCID}_{50}/\text{ml}$, which corresponds to $10^{2.2} \text{CCID}_{50}/2 \text{ mL}$ ($2.2 \log_{10} \text{CCID}_{50} / 2 \text{ ml}$). Control Product (CP) was vaccine diluent (saline solution) and was administered as 4.0 mL (2.0 mL IM + 2.0 mL IN)

1000

1005

1010

At day 0, piglets were vaccinated with the IVP or CP as described. Piglets of T01 and T02 groups were injected intramuscularly in the right side of the neck. Piglets of T01 and T03 groups were administered intranasally, delivering 1.0 mL in each nostril.

1015 Challenge Administration

All pigs were challenged intranasal (IN) with a total challenge volume of 2.0 mL by instilling 1.0 mL of challenge material in each nostril with the Olot/91 virus at a titre of 105.7 CCID₅₀/2 mL.

1020 Results- Viremia

All pigs were RT-qPCR PRRSV negative in serum before vaccination (D0) and before challenge (D181). After challenge, 100% of pigs from the T01 group became viremic at day 185 (3 days post-challenge, DC+3) and remained positive until the end of the study. In the vaccinated groups, 100% (T02 group) and 88% (T03) of the pigs were also positive at DC+3. However, the percentage of positive pigs in both vaccinated groups decreased in time and became significantly lower at DC+8 and day of necropsy (DN) compared to the control group T01.

In both vaccinated groups, the amount of viral load detected in serum was also significantly reduced compared to group T01 at all sampling days post-challenge. At day 185 (DC+3), a significant reduction on viral titres was also observed in the group T03 in relation to T02. Table 1 summarizes the viremia results during the 10-day post-challenge period.

Table 1. Summary of viremia results by group and day of study (challenge phase data)

Group	Study day	N	Log ₁₀ RNA copies / ml of serum					% of viremic animals
			Least Square Means	SE	Range	Lower 95% CB	Upper 95% CB	
T01	D181 (DC-1)	20	1.69	0.18	1.70 to 1.70	1.31	2.08	0.0
T02	D181 (DC-1)	18	1.71	0.18	1.70 to 1.70	1.33	2.09	0.0
T03	D181 (DC-1)	17	1.70	0.26	1.70 to 1.70	1.17	2.22	0.0
T01	D185 (DC+3)	20	6.47	0.18	5.28 to 7.95	6.09	6.85	100.0
T02	D185 (DC+3)	18	5.77	0.18	3.94 to 7.13	5.38	6.15	100.0
T03	D185 (DC+3)	17	5.04	0.26	1.70 to 7.28	4.51	5.57	88.2
T01	D188 (DC+6)	20	5.24	0.18	3.89 to 6.40	4.86	5.62	100.0
T02	D188 (DC+6)	17	3.95	0.18	1.70 to 5.86	3.56	4.34	94.1
T03	D188 (DC+6)	17	3.73	0.26	1.70 to 5.45	3.20	4.26	82.4
T01	D190 (DC+8)	20	4.62	0.18	2.91 to 6.58	4.24	5.00	100.0
T02	D190 (DC+8)	17	2.12	0.18	1.70 to 4.17	1.74	2.51	35.3
T03	D190 (DC+8)	17	2.22	0.26	1.70 to 4.48	1.70	2.75	29.4
T01	D191/192 (DN)	20	5.90	0.18	4.51 to 7.35	5.52	6.28	100.0
T02	D191/192 (DN)	17	2.06	0.18	1.70 to 3.41	1.68	2.45	35.3
T03	D191/192 (DN)	17	1.99	0.26	1.70 to 2.95	1.46	2.51	29.4

N: number; SE: Standard Error; CB: confidence bound; D= day of study; DC: day of challenge; DN:day of necropsy; RT-qPCR positive: > 1.7 log₁₀ RNA copies/ml.

Nasal Shedding

1040 All pigs were RT-qPCR PRRSV negative in nasal swabs before challenge (D181).
After challenge, all pigs from T01 became nasal shedders. In the vaccinated groups, the percentage of pigs that ever shed by the nasal route was 94% (T02 group) and 88% (T03 group). In relation to the T01 group, a significant reduction in the percentage of nasal shedders was observed in the T03 group at days 185, 188 and 191/192 (DC+3, DC+6 and
1045 DN) and in the T02 group at day 191/192 (DN). No significant differences in the percentage of nasal shedders were detected between vaccinated groups. The amount of virus shed by the nasal route was significantly higher in the T01 group compared to the T02 group at DC+6 and DN, and compared to T03 at DC+3, DC+6 and DN. Comparison between vaccinated groups revealed significantly higher viral titres in the T02 group compared to the
1050 T03 at DC+3. Table 2 summarizes the nasal shedding results during the 10-day post-challenge period.

Table 2. Summary of nasal shedding results by group and day of study (post-challenge data)

Group	Study day	N	Least Square Means	Log ₁₀ RNA copies / ml			Upper 95% CB	% of nasal shedders
				SE	Range	Lower 95% CB		
T01	D181 (DC-1)	20	1.70	0.19	1.70 to 1.70	1.32	2.08	0.0
T02	D181 (DC-1)	18	1.68	0.20	1.70 to 1.70	1.28	2.08	0.0
T03	D181 (DC-1)	16	1.69	0.21	1.70 to 1.70	1.27	2.11	0.0
T01	D185 (DC+3)	20	4.61	0.19	3.70 to 5.51	4.23	4.99	100.0
T02	D185 (DC+3)	18	4.66	0.20	1.70 to 6.49	4.26	5.06	88.9
T03	D185 (DC+3)	17	3.35	0.21	1.70 to 5.44	2.94	3.75	70.6
T01	D188 (DC+6)	20	4.36	0.19	3.44 to 5.25	3.98	4.75	100.0
T02	D188 (DC+6)	17	3.33	0.21	1.70 to 5.21	2.92	3.74	94.1
T03	D188 (DC+6)	17	3.02	0.21	1.70 to 5.22	2.62	3.43	76.5
T01	D190 (DC+8)	20	2.21	0.19	1.70 to 3.69	1.83	2.59	40.0
T02	D190 (DC+8)	17	2.39	0.21	1.70 to 4.73	1.98	2.80	35.3
T03	D190 (DC+8)	17	2.21	0.21	1.70 to 3.92	1.80	2.61	35.3
T01	D191/192 (DN)	20	2.55	0.19	1.70 to 5.98	2.17	2.94	50.0
T02	D191/192 (DN)	17	1.81	0.21	1.70 to 3.25	1.40	2.22	11.8
T03	D191/192 (DN)	17	1.82	0.21	1.70 to 2.84	1.41	2.22	11.8

1055 N: number; SE: Standard Error; CB: confidence bound; D= day of study; DC: day of challenge; DN:day of necropsy; RT-qPCR positive: > 1.7 log₁₀ RNA copies/ml

Oral Shedding

All pigs were RT-qPCR PRRSV negative in oral swabs before challenge (D181).

1060 After challenge, the proportion of pigs that ever shed by the oral route was 9/20 (45%), 8/18 (44%) and 7/17 (41%) in T01, T02 and T03 groups, respectively. By the end of the study, all pigs from the T02 group and all but one in the T03 group were negative in oral swabs. In the control group T01, 5/20 were still positive at that time; differences in the percentage of oral shedders between T01 and T02 were statistically significant at the end of study (DN). The amount of virus shed by the oral route was significantly higher in the T01 group compared to the T03 group at day of necropsy, corresponding to 9/10 days post-challenge. Viral load was also significantly higher in the T02 group compared to both T01 and T03 at day 185 (DC+3). Table 3 summarizes the oral shedding results during the 10-day post-challenge period.

1070

Table 3. Summary of oral shedding results by group and day of study (post-challenge data)

Group	Study day	N	Log ₁₀ RNA copies / ml					% of oral shedders
			Least Square Means	SE	Range	Lower 95% CB	Upper 95% CB	
T01	D181 (DC-1)	20	1.70	0.07	1.70 to 1.70	1.57	1.83	0.0
T02	D181 (DC-1)	18	1.70	0.12	1.70 to 1.70	1.45	1.95	0.0
T03	D181 (DC-1)	17	1.70	0.08	1.70 to 1.70	1.54	1.86	0.0
T01	D185 (DC+3)	20	1.79	0.07	1.70 to 2.71	1.66	1.93	10.0
T02	D185 (DC+3)	18	2.17	0.12	1.70 to 5.65	1.92	2.42	27.8
T03	D185 (DC+3)	17	1.79	0.08	1.70 to 2.52	1.64	1.95	11.8
T01	D188 (DC+6)	20	1.85	0.07	1.70 to 2.86	1.72	1.98	15.0
T02	D188 (DC+6)	17	1.95	0.13	1.70 to 2.59	1.70	2.20	23.5
T03	D188 (DC+6)	17	2.11	0.08	1.70 to 3.66	1.95	2.26	35.3
T01	D190 (DC+8)	20	1.70	0.07	1.70 to 1.70	1.57	1.83	0.0
T02	D190 (DC+8)	17	1.75	0.13	1.70 to 1.70	1.49	2.00	0.0
T03	D190 (DC+8)	17	1.70	0.08	1.70 to 1.70	1.54	1.86	0.0
T01	D191/192 (DN)	20	1.95	0.07	1.70 to 3.16	1.81	2.08	25.0
T02	D191/192 (DN)	17	1.73	0.13	1.70 to 1.70	1.48	1.99	0.0
T03	D191/192 (DN)	17	1.72	0.08	1.70 to 2.00	1.56	1.87	5.9

N: number; SE: Standard Error; CB: confidence bound; D= day of study; DC: day of challenge; DN: day of necropsy; RT-qPCR positive: > 1.7 log₁₀ RNA copies/ml

1075 Clinical observations

None of the pigs showed abnormal general condition, depression, respiratory distress, coughing or sneezing during the whole post-challenge period.

1080 Rectal Temperatures

Table 4 summarizes the rectal temperature results during the post-challenge period. In the T01 group, 5/20 pigs (25%) had fever (RT $\geq$ 40.5) at least once during the post-challenge period. In the vaccinated groups T02 and T03, the proportion of pigs that had fever after challenge was 8/18 (44%) and 12/17 (71%), respectively. Rectal temperatures were significantly higher in both vaccinated groups T02 and T03 compared to the T01 group, at day 185 (DC+3).

Table 4. Summary of rectal temperature results in piglets by group and day of study

Group	Study Day	N	Rectal temperatures ($^{\circ}$ C)					% of pigs with fever (RT $\geq$ 40.5)
			LSM	SE	Range	Lower 95% CB	Upper 95% CB	
T01	D182 (DC)	20	39.5	0.09	39 to 40.32	39.3	39.7	0.0
T02	D182 (DC)	18	39.7	0.13	39 to 40.85	39.4	39.9	5.6
T03	D182 (DC)	17	39.7	0.12	38.94 to 40.43	39.4	39.9	0.0
T01	D185 (DC+3)	20	39.9	0.09	39.06 to 40.82	39.7	40.1	5.0
T02	D185 (DC+3)	18	40.3	0.13	39.39 to 41.54	40.1	40.6	33.3
T03	D185 (DC+3)	17	40.4	0.12	39.3 to 41.14	40.1	40.6	52.9
T01	D188 (DC+6)	20	40.0	0.09	39.14 to 40.8	39.8	40.1	25.0
T02	D188 (DC+6)	17	40.1	0.14	39.14 to 41.02	39.9	40.4	29.4
T03	D188 (DC+6)	17	40.2	0.12	39.36 to 41.38	39.9	40.4	29.4
T01	D190 (DC+8)	20	39.5	0.09	38.9 to 40.26	39.3	39.7	0.0
T02	D190 (DC+8)	17	39.7	0.14	39.02 to 40.75	39.4	39.9	5.9
T03	D190 (DC+8)	17	39.6	0.12	39.07 to 40.86	39.4	39.9	17.6
T01	D191 (DC+9)	20	39.5	0.09	38.85 to 39.95	39.3	39.7	0.0
T02	D191 (DC+9)	17	39.7	0.14	39.02 to 40.2	39.4	40.0	0.0
T03	D191 (DC+9)	17	39.7	0.12	38.9 to 41.15	39.5	40.0	5.9

LSM: Least Square Means; SE: Standard Error; CB: confidence bound; RT: Rectal

1090 TemperatureLung lesions

The percentage of lung with lesions for each treatment group is shown in Table 5. Lung visual scores are displayed in Table 6. At necropsy, 18/20 pigs (90%) from the control group T01 had a positive lung visual score, indicating that PRRSV challenge was successful in inducing lung lesions. In the T02 and T03 groups, 12/17 (71%) and 7/16 (44%) pigs scored positive as well. One pig from the T03 group (#300) showed catharral-purulent pleuropneumonia affecting between 10 and 70% of the right lung lobes, between 2 and 30% of the left lung lobes and 70% of the accessory lobe, thus masking the presence of any potential PRRSV-associated lesions. The data from this pig was flagged from the analysis (see flagging report in the MSF). The percentage of lung with lesions observed at necropsy

was significantly higher in the control group T01 compared to the vaccinated groups T02 and T03. No differences were detected between vaccinated groups.

Table 5. Summary of the percentage of lung with lesions

Group	N	Back transformed LSM*	SE	Lower 95% CB	Upper 95% CB	Range
T01	20	3.7 ^A	0.61	2.6	5.0	0.2 to 13.5
T02	17	1.0 ^B	0.35	0.5	1.9	0.0 to 4.4
T03	16	0.7 ^B	0.30	0.2	1.5	0.0 to 5.2

*Different letter superscripts indicate statistical significant difference.

Table 6. Lung visual score by treatment group

Group	Visual score								Total observations
	0		1		2		3		
	(no lesions)		(mild lesions)		(moderate lesions)		(severe lesions)		
	Number	%	Number	%	Number	%	Number	%	Number
T01	2	10.0	17	85.0	1	5.0	0	0.0	20
T02	5	29.4	12	70.6	0	0.0	0	0.0	17
T03	9	56.3	7	43.8	0	0.0	0	0.0	16

Serology (ELISA) results

A summary of the serology results is shown in Table 7. The ELISA results obtained from samples collected at monthly intervals during the vaccination phase are only summarized with descriptive statistics (geometric mean and standard deviations); differences between groups could not be evaluated because there was no replication of the experimental unit (treatments were not comingled). Differences between groups were only tested at study day 181, after animal comingling for the challenge phase. All pigs were ELISA negative prior to vaccination (IDEXX S/P ratio < 0.4). Pigs from the control group T01 remained negative until challenge. One month post-vaccination (Day 28), all pigs from T02 and T03 groups had already developed antibodies to PRRSV and all of them were still positive at challenge (6.5 months post-vaccination). Comparison between groups at day 181 (DC-1) revealed significantly higher least squares mean antibody titers in the T03 group compared to T02 group.

Table 7. Summary of S/P ratio ELISA results,

Treatment Number	Day of Study	N	Geometric Mean/LSM*	SD/SE	Range	% of seropositive
T01	Day 0	20	0.023	0.002	-0.019 to 0.239	0.0
T02	Day 0	18	0.006	0.000	-0.015 to 0.140	0.0
T03	Day 0	17	0.001	0.000	-0.018 to 0.100	0.0
T01	Day 28	20	-0.013	-0.000	-0.025 to 0.018	0.0
T02	Day 28	18	2.487	0.174	2.112 to 2.923	100.0
T03	Day 28	17	2.496	0.218	2.022 to 3.037	100.0
T01	Day 56	20	-0.010	-0.001	-0.079 to 0.182	0.0
T02	Day 56	18	2.366	0.832	-0.078 to 3.591	94.4
T03	Day 56	17	2.523	0.317	1.656 to 3.452	100.0
T01	Day 83	20	0.011	0.000	-0.013 to 0.080	0.0
T02	Day 83	18	2.249	0.328	1.556 to 2.852	100.0
T03	Day 83	17	2.281	0.322	1.582 to 3.162	100.0
T01	Day 113	20	0.042	0.001	-0.003 to 0.091	0.0
T02	Day 113	18	1.598	0.333	0.692 to 2.606	100.0
T03	Day 113	17	1.797	0.360	0.956 to 2.938	100.0
T01	Day 140	20	0.023	0.001	-0.005 to 0.118	0.0
T02	Day 140	18	1.270	0.249	0.612 to 2.076	100.0
T03	Day 140	17	1.461	0.316	0.526 to 2.534	100.0
T01	Day 168	20	0.034	0.001	-0.002 to 0.122	0.0
T02	Day 168	18	1.433	0.329	0.709 to 2.780	100.0
T03	Day 168	17	1.674	0.427	0.677 to 3.175	100.0
T01	Day 181	20	0.055	0.001	0.006 to 0.200	0.0
T02	Day 181	18	1.190	0.060	0.477 to 1.959	100.0
T03	Day 181	17	1.527	0.068	0.665 to 2.422	100.0

*Results from days 0, 28, 56, 83, 113, 140 and 168 are expressed with the Geometric Mean and standard deviation (SD). Day 181 with the Back transformed – Least Square Mean (LSM) and standard error (SE); ELISA positive: S/P ratio ≥ 0.4

Viremia and Shedding

Prior to statistical analysis the RT-qPCR was transformed using an appropriate
 1125 logarithm transformation. The transformed data was analyzed using a general linear
 repeated measured mixed model. Pairwise treatment comparisons were made at each time
 point if the treatment or treatment by time point interaction effect was significant ($P \leq 0.05$).
 Treatment least squares mean and 95% confidence intervals were back transformed for
 presentation. Percentage of animals viremic/shedders was also calculated. Each sample
 1130 was determined to be positive if >50 PRRSV RNA copies/mL ($1.7 \log_{10}$ PRRSV RNA
 copies/mL), which corresponds to a half of the detection limit of the technique (100 PRRSV
 RNA copies/mL). It was also determined if an animal was ever viremic or ever shed for Days
 $\leq$ DC (reference to pre challenge) and Days $>$ DC (post challenge). Frequency tables for

1135 viremia status were calculated for each time point and if the animal was ever viremic for days prior to challenge and days post challenge.

Rectal Temperatures

Rectal temperatures were analyzed using a general linear repeated measures mixed model analysis. Pairwise treatment comparisons were made at each time point if the treatment or treatment by time point interaction effect was significant ($P \leq 0.05$). Treatment least squares means, 95% confidence intervals, the minimum and maximum were calculated for each time point. Descriptive statistics, means, standard deviations, and ranges, were calculated for each treatment and day of study, pre-challenge. Frequency distributions of animals with a fever (rectal temperature $\geq 40.5^\circ\text{C}$) were calculated for each treatment and time point data is collected. It was determined if an animal ever had fever for Days $\leq \text{DC}$ and for Days $> \text{DC}$. Frequency tables for if an animal ever had a fever were calculated for each period, Days $\leq \text{DC}$ and Days $> \text{DC}$.

Serology (ELISA)

1150 Prior to statistical analysis the serology was transformed, where necessary, using an appropriate logarithm transformation. The transformed serology data was analyzed using a general linear repeated measured mixed model. Pairwise treatment comparisons were made at each time point if the treatment or treatment by time point interaction effect was significant ($P \leq 0.05$). Treatment least squares means and 95% confidence intervals were back transformed for presentation. in addition, frequency distributions of the positive/negative results were calculated for each treatment at each time point. Also, it was determined for each animal whether or not it seroconverted (≥ 0.4 S/P ratio) at any time during the study. Frequency distributions of whether or not an animal seroconverted were calculated for each treatment. Descriptive statistics, means, standard deviations, and ranges, were calculated for each treatment, pre-challenge.

Clinical Observations

Frequency distributions of clinical signs prior to challenge and clinical signs following challenge were calculated, separately, for each treatment and time point data was collected. 1165 Frequency distributions of whether an animal ever had a clinical sign, for each phase (Days $\leq \text{DC}$ and Days $> \text{DC}$), was calculated for each treatment.

1170 Body Weights

Body weights were analyzed using a general linear repeated measures mixed model. Least squares means, standard errors, 95% confidence intervals, minimums and maximums were calculated for each treatment at each time point. Pairwise treatment comparisons were made between treatments at each time point if the treatment effect and/or the treatment by time-point interaction was significant. The standard errors and 95% confidence intervals of the estimates were calculated. Descriptive statistics, means, standard deviations, and ranges were calculated for each treatment, pre-challenge. Average daily gain estimates and comparisons were calculated using parameter estimates from the model.

1180

Lung Lesions

Percentage of total lung with lesions was calculated using the following formula: Percentage of total lung with lesions = $(0.10 \times \text{left cranial}) + (0.10 \times \text{left middle}) + (0.25 \times \text{left caudal}) + (0.10 \times \text{right cranial}) + (0.10 \times \text{right middle}) + (0.25 \times \text{right caudal}) + (0.10 \times \text{accessory})$. The arcsine square root transformation was applied to the percentage of total lung with lesions prior to analysis. The transformed lung lesions was analyzed with a general linear mixed model. Pairwise comparisons were made between treatment groups if the treatment effect was significant. Back transformed least squares means of percentage of total lung with lesions, their standard errors, and their 95% confidence intervals were calculated as well as the minimums and maximums.

1190

Lung Lesion Assessment Score

Frequency distributions of lung lesion assessment scores were calculated for each treatment. The scores, normal or not normal, were analyzed using a generalized linear mixed model for binomial data. If the general linear mixed model did not converge, Fisher's Exact test was used to analyze the data. If the treatment main effect was significant, then pairwise treatment comparisons was made.

1195

Models for Analyses

Transformed percentage of total lung with lesions was analyzed with a general linear mixed model with fixed effects, treatment, and random effects pen and block within pen. Linear combinations of the parameter estimates were used in a priori contrasts after testing for a significant ($P \leq 0.05$) treatment effect. Comparisons were made between treatments. The 5% level of significance ($P \leq 0.05$) was used to assess statistical differences. Least

1200

1205 squares means (back-transformed), standard errors, 95% confidence intervals of the means and ranges were calculated for each treatment.

Viremia status was analyzed with a general linear repeated measures mixed model with a logit link with fixed effects: treatment, time point, and treatment by time point
1210 interaction, and random effects: pen, block within pen, and animal within block, pen, and treatment, which is the animal term. Linear combinations of the parameter estimates were used in a priori contrasts after testing for a significant ($P \leq 0.05$) treatment effect or treatment by time point interaction. Comparisons were made between treatments at each time point. The 5% level of significance ($P \leq 0.05$) was used to assess statistical differences. Least
1215 squares means (back transformed), standard errors, and 95% confidence intervals of the means were calculated for each treatment and time point. if the model did not converge, Fisher's Exact were used for the analysis.

Lung assessment scores, normal/not normal, and if ever viremic were analyzed with
1220 a generalized linear mixed model with fixed effects treatment, and random effects pen and block within pen, via GLIMMIX. If the treatment main effect was significant, then pairwise treatment comparisons were made. If GLIMMIX did not converge, Fisher's Exact test was used for analysis.

1225 Viral load, serology, body weight, and rectal temperature were analyzed with a generalized linear repeated measures mixed model with fixed effects: treatment, time point, and treatment by time point interaction, and random effects: pen, block within pen, and animal within block, pen, and treatment, which is the animal term. Linear combinations of the parameter estimates were used in a priori contrasts after testing for a significant
1230 ($P \leq 0.05$) treatment effect or treatment by time point interaction. Comparisons were made between treatments at each time point. The 5% level of significance ($P \leq 0.05$) was used to assess statistical differences. Least squares means (back transformed for viral load and serology), standard errors, 95% confidence intervals of the means and ranges were calculated for each treatment and time point. All hypothesis tests were conducted at the
1235 0.05 level of significance using two-sided tests.

Discussion

The objective of the present study was thus to evaluate the Duration of Immunity (DOI) of the EU PRRS modified live vaccine (SEQ ID NO:5) in pigs vaccinated at 1 day of
1240 age by the intramuscular (IM, group T02) or the intranasal (IN, group T03) route, upon

inoculation with a genotype I PRRS isolate as a respiratory challenge at 26 weeks post-vaccination. The primary variable in determining the efficacy was viral load in serum (viremia) of treatment groups T02 and T03 in comparison to T01 pigs. Lung lesions, rectal temperatures, shedding, clinical signs and body weight were compared as secondary variables.

The test was valid since all T01 pigs remained PRRSV viro-negative throughout the vaccination period and no confounding disease factors were detected. A protective effect of both IM and IN vaccination was observed when comparing viral load in serum between groups; the control group T01 had significantly higher viral titers compared to the vaccinated groups T02 and T03 at all sampling days post-challenge. In addition, in relation to the control group, the proportion of viremic pigs was also significantly reduced at days 8 and 9/10 post-challenge in both vaccinated groups. When vaccinated groups were compared, significantly lower viral titers were detected at day 185 (DC+3) in the group vaccinated by the IN route (T03) compared to the group vaccinated intramuscularly (T02). The benefit of both IM and IN vaccination on virus replication was also supported by the significant reduction observed in both vaccinated groups in the amount of virus shed by the nasal route as well as in the percentage of nasal shedders. In addition, a positive effect of vaccination was also observed on oral shedding at 9/10 days post-challenge, as demonstrated by the significant reduction in viral load (T03 group) or the percentage of positive pigs in oral swabs (T02 group) in relation to the control group.

After challenge, rectal temperatures were significantly higher in both vaccinated groups compared to the control group at DC+3. At that time, the proportion of pigs with fever ($RT \geq 40.5^{\circ}\text{C}$) in the groups vaccinated with the IVP by the IM and the IN route was 6/18 (33%) and 9/17 (53%), respectively. In the control group, only 1/20 (5%) had fever at DC+3. However, it is noteworthy to mention that at that time (DC+3), the viral load detected in serum was indeed significantly reduced in both vaccinated groups compared to the control group, thus suggesting that PRRSV replication was probably not the sole reason behind the elevated rectal temperatures observed in vaccinated pigs shortly after challenge. In addition, from then onwards, rectal temperatures from vaccinated pigs decreased and, by day 8 post-challenge, mean rectal temperatures in all groups were already below 40°C .

Besides elevated rectal temperatures, none of the pigs from any group showed clinical signs compatible with PRRSV (abnormal body condition, depression, respiratory

distress, coughing or sneezing) during the whole post-challenge period. In addition, no differences in body weight or average daily weight gain were detected between groups.

At necropsy, 18/20 pigs (90%) from the control group T01 had a positive lung visual score, indicating that PRRSV challenge was successful in inducing lung lesions. In the T02 and T03 groups, 12/17 (71%) and 7/16 (44%) pigs scored positive as well. Comparison between treatment groups showed significant higher % of lung with lesions in the control group than in the vaccinated groups, demonstrating the benefit of vaccination on reducing the PRRSV-associated lung lesions regardless of the administration route.

Serology data demonstrated that vaccination with the IVP by either the IM or the IN route induced the development of PRRSV-specific antibodies within 28 days post-vaccination. All vaccinated pigs were seropositive to PRRSV antibodies at challenge (6.5 months post-vaccination), indicating that both administration routes were able to elicit a strong and protective antibody response to vaccination. However, comparison between vaccinated groups showed significantly higher antibody titers in the group vaccinated by the IN route compared to the group vaccinated by the IM route.

Although vaccination by both the IN and the IM routes conferred a clear protection against PRRSV challenge at 26 weeks post-vaccination, comparison between groups revealed that at 3 days post-challenge, the group vaccinated by the IN route (T03) had significantly lower viremia, nasal shedding and oral shedding compared to the group vaccinated intramuscularly (T02). These results indicate that, under the conditions of the present study, the protection obtained following the IN vaccination was stronger than following IM vaccination. These differences in the virological outcome would be explained by the significantly higher antibody titres detected by ELISA before challenge in the group vaccinated IN (S/P ratio range: 0.665-2.422), compared to the group vaccinated IM (S/P ratios range: 0.477-1.959).

Vaccination with a single administration of the EU PRRS MLV vaccine (SEQ ID NO:5) containing a dose of 2.5 log₁₀ CCID₅₀ to 1 day-old seronegative pigs by either the IM or the IN route conferred a duration of immunity of 26 weeks, as seen by the significant reduction on the viral load detected in serum after challenge with a pathogenic EU PRRSV strain at 26 weeks post-vaccination. Efficacy was also supported by the significant reduction on the percentage of lung lesions at necropsy, as well as the reduction of nasal and oral shedding.

Laboratory Methods

1315 In regard of RT qPCR, viremia (PRRSV load in serum) and shedding (PRRSV load
in swab material) were measured by means of a Reverse Transcription (RT) qPCR
performed following local SOP on the serum samples collected before vaccination (D-1),
before challenge (D62) and at 3, 5, 7 and 10 days post-challenge (D66, D68, D70 and D73).
In brief, the purified viral RNA was used as template, reverse transcribed at 50°C for 30
minutes, and denatured at 95°C for 5 minutes. The PCR program of reactions consisted of
1320 40 cycles of denaturation at 95°C for 20 seconds and annealing at 53°C for 40 seconds.
The qRT-PCR was conducted in a 7500 Real-Time PCR System thermalcycler. Appropriate
primers and probe were selected for Lelystad virus as follows:

Forward primer (Lelystad F): 5'-GCACCACCTCACCCAGAC-3' (SEQ ID NO: 6, final
1325 concentration 0.5 micromolar).

Reverse primer (Lelystad R): 5'-CAGTTCCTGCGCCTTGAT-3' (SEQ ID NO:7, final
concentration 0.5 micromolar).

Probe (Lelystad S): 5'-6-FAM- CCTCTGCTTGCAATCGATCCAGAC –TAMRA-3'
(SEQ ID NO:8, final concentration 0.6 micromolar).

1330

Serology (ELISA)

Sera collected before vaccination (D0), before challenge (D67) and at necropsy
(D77) were tested for antibodies to PRRSV using the IDEXX PRRS X3 ELISA, following the
manufacturer's instructions. In brief, serum samples were diluted 1:40 in 96-well plate
1335 coated with a recombinant PRRSV antigen and incubated 30 minutes at 18-26°C. After
washing, an anti-porcine horseradish peroxidase conjugate was added and plates were
incubated for another 30 minutes at 18-26°C. Unbound conjugate was washed away and
TMB substrate was added to the wells. Color development was measured at 650 nm. S/P
values were recorded for each sample. If the S/P ratio was ≥ 0.4 the sample was classified
1340 as positive for PRRS antibodies. If the S/P ratio was < 0.4 the sample was classified as
negative. ELISA instructions are included in the study file.

Example 4 - Onset of immunity (OOI) of a Modified Live EU PRRSV Vaccine administered
to 2 weeks old pigs against challenge with an European PRRS isolate at 4 weeks post-
1345 vaccination

The objective of this study was to evaluate the Onset of Immunity (OOI) of an experimental EU PRRSV MLV vaccine (the SEQ ID NO:5 virus) administered to 2-week old pigs for the prevention of respiratory disease induced by PRRSV at 4 weeks post-vaccination. The primary variable in determining prevention of disease was viral load in serum in treatment group T02 (vaccinated with the Test Item) compared to T01 (vaccinated with control product), supported by lung lesions, oral and nasal shedding, clinical observations, rectal temperatures and body weight.

At the age of 14-15 days, a single 2.0 mL dose of the Control Product (CP) or the Test Item (TI, SEQ ID NO:5 virus) was administered as an intramuscular injection to each animal. Four weeks post-vaccination, pigs were challenged intranasally with the EU PRRSV isolate Olot/91 as a respiratory challenge. During the challenge phase, blood samples, nasal and oral swabs, clinical observations and rectal temperatures were collected every 2-3 days until day of necropsy. Pigs were weighed the day before vaccination, before challenge and at necropsy. Ten days post-challenge, pigs were anesthetized, euthanized and necropsied. Upon necropsy, lungs were evaluated for the presence of PRRSV lesions and scored. This study was valid since T01 pigs remained PRRSV sero and viro-negative throughout the vaccination period and no confounding disease factors were detected.

A protective effect of vaccination was observed when comparing viral load in serum between groups. The group vaccinated with the TI (T02) had significantly lower viral titers compared to the control group at all sampling days post-challenge.

Efficacy was also supported by the significant reduction of the percentage of lung with lesions at necropsy (10 days post-challenge), the reduction on nasal and oral shedding, as well as by the reduction on rectal temperatures at 7 days post-challenge. No differences were detected in body weight. Vaccination induced the development of PRRSV-specific antibodies within 4 weeks post-vaccination. Ten days after challenge, antibody titers remain significantly higher in the T02 group compared to the control one (T01). In conclusion, a single intramuscular administration of the experimental EU PRRSV MLV vaccine containing a dose of $2.0 \log_{10}$ CCID₅₀ to 14-15 day-old pigs (seronegative to PRRSV) was protective against respiratory disease induced after challenge with a pathogenic EU PRRS strain at 4 weeks post-vaccination. Experimental Design is as follows:

Group	VACCINATION (Day 0)		CHALLENGE (Day 27)		N
	Test material	Dose	Test material	Dose	
T01	Control product	2 mL	PRRSV Olot/91	5.4 log ₁₀ CCID ₅₀ /2 mL	20
T02	Test Item	2.0 log ₁₀ CCID ₅₀ /2 mL	PRRSV Olot/91	5.4 log ₁₀ CCID ₅₀ /2 mL	20

At Day 27, after re-housing of the pigs, all animals were challenged with 2 ml of the challenge strain Olot/91, by intranasal (IN) route. The pigs were challenged with a total challenge volume of 2.0 mL by instilling 1.0 mL challenge material in each nostril. Pigs were held in a head up position for some seconds after challenge.

Clinical observations and rectal temperatures

Rectal temperatures and clinical observations were recorded on the days specified in **Error! Reference source not found.** Clinical observations included general condition, depression, sneezing, coughing and respiratory distress. Rectal temperatures were collected according to local standard procedures. Those days (before and after vaccination/challenge inoculation) in which a complete clinical examination was not performed, the general health of the pigs was observed and recorded in Daily Review Animal Care (DRAC) forms following standard SOP.

Necropsy and Lung Scoring.

Upon necropsy, lung lesions were scored using the following methods: 1) the percentage of consolidation for each lobe (left cranial, left middle, left caudal, right cranial, right middle, right caudal and accessory) will be scored and recorded as percent of lobe observed with lesions; and 2) a numeric visual score (0, 1, 2, 3).

RT qPCR

Viremia (PRRSV load in serum) and shedding (PRRSV load in nasal and oral swabs) were measured by means of a Reverse Transcription (RT) qPCR performed following standard procedures. In brief, the purified viral RNA was used as template, reverse transcribed at 50°C for 30 minutes, and denatured at 95°C for 5 minutes. The PCR program of reactions consisted of 40 cycles of denaturation at 95°C for 20 seconds and annealing at 53°C for 40 seconds. The qRT-PCR was conducted in a 7500 Real-Time PCR System thermalcycler. Results were expressed as RNA copies/ml.

1405 Serology

Sera collected before vaccination, before challenge and at necropsy were tested for antibodies to PRRSV using the IDEXX PRRS X3 ELISA, following the manufacturer's instructions. In brief, serum samples were diluted 1:40 in 96-well plate coated with a recombinant PRRSV antigen and incubated 30 minutes at 18-26°C. After washing, an anti-porcine horseradish peroxidase conjugate was added and plates were incubated for another 30 minutes at 18-26°C. Unbound conjugate was washed away and TMB substrate was added to the wells. Color development was measured at 650 nm. S/P values were recorded for each sample. If the S/P ratio was ≥ 0.4 the sample was classified as positive for PRRS antibodies. If the S/P ratio was < 0.4 the sample was classified as negative. ELISA instructions are included in the study file. The study ended at day 37 (euthanasia).

Viremia and Shedding

Prior to statistical analysis the RT-qPCR data was transformed using an appropriate logarithm transformation. The transformed data was analyzed using a general linear repeated measured mixed model. Pairwise treatment comparisons were made at each time point if the treatment or treatment by time point interaction effect is significant ($P \leq 0.05$). Treatment least squares mean and 95% confidence intervals were back transformed for presentation. Percentage of days viremic/shedding was also calculated. Each sample was determined to be positive if >250 PRRSV RNA copies/mL, which corresponded to a half of the detection limit of the technique (500 PRRSV RNA copies/mL of sample). It was also determined if an animal was ever viremic or ever shed for Days ≤ 27 (reference to pre challenge) and Days >27 (post challenge).

Lung Lesions

Percentage of total lung with lesions was calculated using the following formula:
Percentage of total lung with lesions = $(0.10 \times \text{left cranial}) + (0.10 \times \text{left middle}) + (0.25 \times \text{left caudal}) + (0.10 \times \text{right cranial}) + (0.10 \times \text{right middle}) + (0.25 \times \text{right caudal}) + (0.10 \times \text{accessory})$. The arcsine square root transformation was applied to the percentage of total lung with lesions prior to analysis. The transformed lung lesions were analyzed with a general linear mixed model. Pairwise comparisons were made between treatment groups if the treatment effect was significant. Back transformed least squares means of percentage of total lung with lesions, their standard errors, and their 95% confidence intervals were calculated as well as the minimums and maximums. Frequency distributions of lung lesion assessment scores were calculated for each treatment. The scores, normal or not normal,

1440 were analyzed using a general linear mixed linear model for binomial data. If the general linear mixed model did not converge, Fisher's Exact test was used to analyze the data. If the treatment main effect was significant, then pairwise treatment comparisons was made.

Lung scoring

1445 The percentage of lung with lesions for each treatment group is shown in Table 8. Lung visual scores are displayed in Table 9. At necropsy, 17/18 pigs (94.4%) from the control group T01 had a positive lung visual score indicating that PRRSV challenge was successful in inducing lung lesions. In the T02 group, 14/19 (73.7%) pigs scored positive as well. Control group T01 had a significantly higher LS mean percentage of lung with lesions compared to the T02 group. Regarding the total lung visual score, lungs from all T02 pigs were scored as either 0 (no lesions) or 1 (mild lesions). In the T01 control group, 7/18 (38.9%) pigs were given a visual score of 2 (moderate lesions). None of the lungs from any group was given a visual score of 3 (severe lesions).

Table 8. Summary of the percentage of lung with lesions

	N	Back transformed LS Mean	Standard Error	Lower 95% CL	Upper 95% CL	Range
T01	18	7 ^a	2	4	11	0 to 29
T02	19	2 ^b	1	1	5	0 to 10

Different superscripts mean significant differences among groups.

1455

Table 9. Lung visual score by treatment group

Treatment	Visual score						Total observations
	0 (no lesions)		1 (mild lesions)		2 (moderate lesions)		
	Number	%	Number	%	Number	%	Number
T01	1	5.6	10	55.6	7	38.9	18
T02	4	26.3	14	73.7	0	0.0	19

RT-qPCR results, Viremia (PRRSV load in serum)

1460 All pigs were found RT-qPCR PRRSV negative in blood at day 0. At challenge (day 27), PRRSV RNA could be detected in 100% of the pigs vaccinated with the T1 (T02 group). By that time, all T01 pigs were still PRRSV negative. In control pigs, viremia was first detected at day 29 (2 days post-challenge) and pigs remained PRRSV positive until the end of the study. At all sampling days post-challenge, viral loads detected in control T01 group were significantly higher than those detected in the T02 group. Table 10 summarizes the

1465 viremia results from day 27 (day of challenge) until day 36. Summary of Least square means and LS Means differences between groups are shown in Table 11.

Table 10. Summary of viremia results by group and day of study.

	Day of Study	Log ₁₀ RNA copies / ml of serum					% of viremic pigs
		Least Squares Mean	Standard Error	Range	Lower 95% Confidence Bound	Upper 95% Confidence Bound	
T01	Day 27	2.4	0.16	2.4 to 2.4	2.0	2.8	0.0%
T02	Day 27	6.0	0.16	4.6 to 7.8	5.6	6.4	100.0%
T01	Day 29	7.4	0.25	5.3 to 9.4	6.8	7.9	100.0%
T02	Day 29	5.6	0.25	3.8 to 7.8	5.1	6.1	100.0%
T01	Day 31	8.1	0.27	6.3 to 9.2	7.5	8.6	100.0%
T02	Day 31	6.9	0.27	4 to 8.9	6.4	7.5	100.0%
T01	Day 34	7.3	0.24	5.4 to 8.4	6.8	7.8	100.0%
T02	Day 34	6.4	0.24	4.2 to 8	5.9	6.9	100.0%
T01	Day 36	7.2	0.28	5.7 to 8.6	6.6	7.7	100.0%
T02	Day 36	5.6	0.28	2.7 to 8.4	5.0	6.2	100.0%

RT-qPCR positive: > 2.4 log₁₀ RNA copies/ml

Table 11. Summary table of Least Square Means for viremia results.

Treatment Number	Least Square Mean at different study dates				
	Day 27	Day 29	Day 31	Day 34	Day 36
T01	2.4 ^a	7.4 ^a	8.1 ^a	7.3 ^a	7.2 ^a
T02	6.0 ^b	5.6 ^b	6.9 ^b	6.4 ^b	5.6 ^b

Different superscripts mean significant differences among groups.

1470

Nasal and oral shedding

1475 All pigs were found RT-qPCR PRRSV negative in nasal and orals swabs at day 0. At challenge (day 27), 70% and 85% of pigs vaccinated with the TI (T02 group) had detectable PRRSV RNA in nasal and oral swabs, respectively. None of the pigs from the T01 group shed at that time. During the post-challenge period, the percentage of nasal shedders in the T01 group ranged from 85% (Day 34) to 100% (Day 29). In the T02 group, percentages of nasal shedders ranged from 42% (Day 36) to 90% (Day 29). Regarding oral shedding, the percentage of positive pigs ranged between 95 to 100% in the T01 group and between 73.7 to 100% in group T02. Comparison of LSM between groups revealed

1480 significant higher viral loads in T01 group at all days post-challenge but day 34 for nasal swabs and at days 31 and 36 for oral swabs.

Results on nasal and oral shedding from day 27 (day of challenge) until day 36 are summarized in Table 12 and

1485

1490 **Table 54**, respectively. Summary of LSM and LS Means differences between groups are shown in Tables 13 and 15.

Table 12. Summary of nasal shedding results by group and day of study.

	Day of Study	RNA copies / ml (per swab)					% of shedding pigs
		Least Squares Mean	Standard Error	Range	Lower 95% Confidence Bound	Upper 95% Confidence Bound	
T01	Day 27	2.4	0.19	2.4 to 2.4	2.0	2.8	0.0%
T02	Day 27	3.5	0.26	2.4 to 4.9	3.0	4.1	70.0%
T01	Day 29	6.5	0.19	5.4 to 7.6	6.1	6.9	100.0%
T02	Day 29	4.8	0.26	2.4 to 6.8	4.2	5.3	90.0%
T01	Day 31	6.4	0.19	5.2 to 7.7	6.0	6.8	100.0%
T02	Day 31	5.0	0.27	2.4 to 6.8	4.5	5.5	94.7%
T01	Day 34	4.5	0.19	2.4 to 6.9	4.1	4.9	85.0%
T02	Day 34	4.2	0.27	2.4 to 7.2	3.7	4.8	84.2%
T01	Day 36	4.7	0.20	2.4 to 6.5	4.3	5.1	88.9%
T02	Day 36	3.3	0.27	2.4 to 5.6	2.8	3.9	42.1%

RT-qPCR positive: > 2.4 log₁₀ RNA copies/ml

Table 13. Summary table of Least Square Means for nasal shedding results.

Treatment Number	Least Square Mean at different study dates				
	Day 27	Day 29	Day 31	Day 34	Day 36
T01	2.4 ^a	6.5 ^a	6.4 ^a	4.5 ^a	4.7 ^a

Treatment Number	Least Square Mean at different study dates				
	Day 27	Day 29	Day 31	Day 34	Day 36
T02	3.5 ^b	4.8 ^b	5.0 ^b	4.2 ^a	3.3 ^b

1495

1500

Table 5. Summary of oral shedding results by group and day of study.

	Day of Study	RNA copies / ml (per swab)					% of shedding pigs
		Least Squares Mean	Standard Error	Range	Lower 95% Confidence Bound	Upper 95% Confidence Bound	
T01	Day 27	2.4	0.19	2.4 to 2.4	2.0	2.8	0.0%
T02	Day 27	4.9	0.26	2.4 to 7.4	4.3	5.4	85.0%
T01	Day 29	4.2	0.19	2.4 to 5.9	3.8	4.6	95.0%
T02	Day 29	4.0	0.26	2.4 to 5.2	3.5	4.6	90.0%
T01	Day 31	5.2	0.19	4 to 6.9	4.8	5.6	100.0%
T02	Day 31	4.4	0.26	2.4 to 6.6	3.8	4.9	94.7%
T01	Day 34	4.9	0.19	3.7 to 6.7	4.5	5.3	100.0%
T02	Day 34	4.6	0.26	3 to 5.8	4.1	5.1	100.0%
T01	Day 36	4.6	0.20	3.5 to 6.1	4.2	5.0	100.0%
T02	Day 36	3.8	0.26	2.4 to 5.2	3.3	4.4	73.7%

RT-qPCR positive: > 2.4 log₁₀ RNA copies/ml

Table 6. Summary table of Least Square Means for oral shedding results.

Treatment Number	Least Square Mean at different study dates				
	Day 27	Day 29	Day 31	Day 34	Day 36
T01	2.4 ^a	4.2 ^a	5.2 ^a	4.9 ^a	4.6 ^a
T02	4.9 ^b	4.0 ^a	4.4 ^b	4.6 ^a	3.8 ^b

Different superscripts mean significant differences among groups.

1505 Serology

All pigs were serologically negative for PRRSV prior to vaccination (IDEXX S/P ratio<0.4). At challenge (4 weeks post-vaccination), all control pigs were still seronegative, while 90% of the pigs vaccinated with the TI had seroconverted to PRRSV (IDEXX S/P ratio > 0.4). At day 36 (9 days post challenge), all pigs were seropositive to PRRSV. Pigs from group T02 had significantly higher LS mean antibody titres compared to the control group T01. Table 76 summarizes the serology results obtained by ELISA.

Table 76. S/P ratio ELISA results (LS mean and range) and % of seropositive pigs by treatment group and day

Treatment	Day 27		Day 36	
	LS Mean (range)	% seropositive	LS Mean (range)	% seropositive
T01	0.011 ^a (-0.087 to 0.123)	0.0%	1.582 ^a (0.846 to 2.357)	100%
T02	1.617 ^b (0.31 to 2.561)	90%	1.960 ^b (0.762 to 2.884)	100%

Different superscripts mean significant differences among groups.

Discussion

This study evaluated the Onset of Immunity (OOI) of a modified live PRRSV vaccine in pigs vaccinated at 14-15 days of age upon inoculation with a pathogenic genotype I PRRSV isolate as a respiratory challenge at 4 weeks after vaccination. The test was valid since all T01 pigs remained PRRSV sero and viro-negative throughout the vaccination period and no confounding disease factors were detected.

The primary variable in determining the efficacy was viral load in serum of treatment group T02 in comparison to T01 pigs. Lung lesions, rectal temperatures, shedding, clinical signs and body weight were compared as secondary variables.

A protective effect of vaccination was observed when comparing viral load in serum between T01 and T02 groups; the group vaccinated with the TI had significantly lower viral titers compared to the control group T01 at all sampling days post-challenge.

After challenge, oral and nasal shedding could be detected in all pigs regardless of the treatment group. However, significant higher viral loads were detected in the T01 group, at all days but day 34 for nasal swabs and at days 31 and 36 for oral swabs.

Regarding lung lesions, a protective vaccine effect was also observed for the T02 pigs when comparing back-transformed least squares mean percentage of lung lesions between groups. In addition, all 14 T02 pigs that were given a positive visual score at necropsy were scored as 1 (mild lesions). In contrast, lungs from the 17 pigs in T01 group that also scored positive, 10 were given a score of 1 (mild lesions) and 7 a score of 2 (moderate lesions).

During the post-challenge period, rectal temperatures were significantly lower in pigs vaccinated with the TI at day 7 post-challenge. By that time, 20% of the T01 pigs had fever, whereas none in the T02 group did. Before challenge, rectal temperatures in the T02 group were also significant lower compared to T01. However, none of the pigs had fever and there was no apparent clinical condition in that group that might have induced an increase in rectal temperature. Besides increased rectal temperatures, no other clinical observations compatible with PRRS (depression, respiratory distress, coughing or sneezing) were observed in any pig during the whole time-period. In addition, no differences in body weight could be detected at any time point between groups.

Serology data demonstrated that vaccination with the TI (T02 group) induced the development of PRRSV-specific antibodies within 4 weeks post-vaccination. Antibody titers in the T02 group increased until the end of the study and were significantly higher than titers detected by that time in the non-vaccinated challenged group T01.

Vaccination with a single intramuscular administration of the experimental EU PRRSV MLV vaccine containing a dose of $2.0 \log_{10} \text{CCID}_{50}$ to 14-15 day-old pigs was protective against PRRSV, as seen by the significant reduction on the viral load detected in serum after challenge with a pathogenic EU PRRS strain at 4 weeks post-vaccination. Efficacy was also supported by the protective effect observed on the percentage of lung lesions at necropsy (10 days post-challenge), reduction on oral and nasal shedding as well as reduction on rectal temperatures at 7 days post-challenge

Example 5- Assessment of the potential effect of maternally-derived antibodies on the efficacy of the Modified Live EU PRRSV Vaccine administered to 1-day-old seropositive pigs against challenge with a European PRRSV isolate

The objective of the present study was to evaluate the influence of maternally derived antibodies (MDA) on the efficacy of the EU PRRS MLV (the virus expressed from

SEQ ID NO:5), when administered in 1 day-old piglets by the intramuscular (IM, group T02)
1565 or the intranasal (IN, group T03) route.

The study was designed based on the recommendations of the
EMA/CVMP/WP/439467/2007: "Reflection paper on the demonstration of a possible impact
of maternally derived antibodies on vaccine efficacy in young animals" and the European
Pharmacopoeia (Ph. Eur.) Monograph 04/2008:50207: "Evaluation of efficacy of veterinary
1570 vaccines and immunosera". Both documents recommend that the influence of passively
acquired and maternally derived antibodies on the efficacy of a vaccine has to be
adequately evaluated. In addition, EMA/CVMP/WP/439467/2007 states that the efficacy of
the vaccine in animals vaccinated in the presence of MDAs should be, notwithstanding
normal biological variation, similar to that obtained in animals of the same age but
1575 vaccinated in the absence of MDAs.

Based on the abovementioned specifications, the onset of protective Immunity (OOI)
of the modified live EU PRRSV vaccine was evaluated in seropositive pigs vaccinated (IM
and IN routes) at 1 day of age upon inoculation with a pathogenic genotype I PRRSV isolate
as a respiratory challenge. The animals were challenged at the time when the levels of
1580 MDAs detected by seroneutralization test (SNT) in the control group (T01) became
undetectable. To demonstrate protection against challenge, the same parameters evaluated
in a previous study performed in seronegative animals were evaluated (viral load in serum,
lung lesions, rectal temperatures, shedding, clinical signs and body weight).

The test was valid since all control pigs (T01 group) remained PRRSV viro-negative
1585 throughout the vaccination period and no confounding disease factors were detected.

When the EU PRRS MLV was administered by the IM route, a protective effect of
vaccination was observed as seen by a significant reduction of viral load in serum compared
to the control group; efficacy was also supported by the significant reduction of nasal and
oral shedding as well as significant reduction in rectal temperatures. Differences in mean
1590 percentage of lung lesions were close to significance ($p=0.092$). The efficacy observed
following the IM administration in the present study is similar to that obtained in a previous
study, in which the efficacy was evaluated in seronegative animals; in both studies, a
significant impact of vaccination was observed on the primary variable (reduction on
viremia) and also supported by a reduction on nasal shedding and rectal temperatures.

1595 When the EU PRRS MLV was administered by the IN route, no protection was achieved against PRRSV challenge, as seen by the lack of significant differences in any of the parameters evaluated.

1600 In conclusion, the results of the present study demonstrate lack of interference of maternally derived antibodies with vaccine efficacy, when the vaccine is administered intramuscularly in 1 day old piglets. However, when the vaccine is administered by the IN route, an immune response was not generated in most pigs following vaccination, presumably due to neutralization of the vaccine by MDA. To produce PRRSV MDA positive piglets, six pregnant sows were vaccinated with the EU PRRS MLV vaccine lot VMRD13-015 (5 log₁₀ CCID₅₀/2 mL) during the first half of gestation (45 days of pregnancy). The day before the expected farrowing date, parturition was induced with an intramuscular injection of cloprostenol (Cyclix® Porcino, Virbac). All sows farrowed the next day. All sows were seropositive to PRRS at day 0. Results are shown

1610 Vaccine virus was provided at a potency of 6.0 log₁₀CCID₅₀ /ml. At day 0, the IVP was diluted with vaccine diluent (lot T22019) to match the target titer (2.5 log₁₀ CCID₅₀/2 mL). One aliquot of the IVP was collected for titration on BHK-21-C12-26 cells to confirm the dosage. An additional IVP sample was frozen (-80±10°C) and stored as a retention sample. Titration on BHK-21-C12-26 cells was performed following local standard procedures. The reconstituted and diluted vaccine had a titer of 101.8 CCID₅₀/ml, which corresponds to 102.1 CCID₅₀/2 mL (2.1 log₁₀CCID₅₀ /2 ml)

1615 At day 0, piglets were vaccinated with the IVP or CP as described in section 4. Piglets of T01 and T02 groups were injected intramuscularly in the right side of the neck. Piglets of T01 and T03 groups were administered intranasally, delivering 1.0 mL in each nostril. The challenge material was the genotype 1 Spanish PRRSV isolate Olot/91, grown in Porcine Alveolar Macrophages (PAM). This strain was isolated in 1991 from a case of late-term abortion in sows.

Viremia

1625 All pigs were found RT-qPCR PRRSV negative in serum before vaccination (D0) and all pigs from the T01 group remained so until challenge. In contrast, 8/16 (50%) piglets from the T02 group (IM vaccinated) and 1/19 (5.3%) piglets from T03 group (IN vaccination) were RT-qPCR PRRSV positive at challenge (67 days post-vaccination).

After challenge, 100% of pigs from the T01 group became viremic at D70 (3 days post-challenge) and remained positive until the end of the study (DC+10). In the vaccinated groups (T02 and T03), all pigs were detected PRRSV positive at least once; however, by the end of the study (DC+10), only 11/16 T02 pigs (68.8%) were still viremic. In contrast, all
 1630 T03 pigs but one were positive at all sampling points post-challenge.

Pigs from the T02 group had significantly lower viral load in serum than pigs from the T01 control group at all sampling days post-challenge. At DC+3 and DC+10, viral load in T02 group was also significantly lower than in the T03 group. No significant differences were detected between T01 and T03 groups.

1635 Table 7 summarizes the viremia results during the 10-day post-challenge period.

Table `17. Summary of viremia results by group and day of study (post-challenge data)

Group	Study day	N	Log ₁₀ RNA copies / ml of serum					% of viremic animals
			Least Square Means	SE	Range	Lower 95% CB	Upper 95% CB	
T01	D67 (DC)	18	1.65	0.20	1.70 to 1.70	1.24	2.05	0.0
T02	D67 (DC)	16	2.25	0.36	1.70 to 3.84	1.53	2.97	50.0
T03	D67 (DC)	19	1.82	0.29	1.70 to 3.80	1.25	2.40	5.3
T01	D70 (DC+3)	18	6.60	0.20	5.23 to 7.66	6.20	7.01	100.0
T02	D70 (DC+3)	16	2.87	0.36	1.70 to 6.59	2.15	3.59	50.0
T03	D70 (DC+3)	19	6.30	0.29	1.70 to 8.10	5.72	6.87	94.7
T01	D73 (DC+6)	18	6.39	0.20	4.87 to 7.44	5.99	6.80	100.0
T02	D73 (DC+6)	16	5.18	0.36	1.70 to 7.00	4.45	5.90	93.8
T03	D73 (DC+6)	19	5.80	0.29	1.70 to 7.70	5.22	6.37	94.7
T01	D75 (DC+8)	18	5.32	0.20	3.32 to 7.21	4.92	5.73	100.0
T02	D75 (DC+8)	16	4.18	0.36	2.25 to 6.22	3.46	4.90	100.0
T03	D75 (DC+8)	19	5.07	0.29	1.70 to 7.13	4.50	5.65	94.7
T01	D77 (DC+10)	18	5.29	0.20	3.51 to 6.47	4.89	5.70	100.0
T02	D77 (DC+10)	16	2.96	0.36	1.70 to 5.86	2.23	3.68	68.8
T03	D77 (DC+10)	19	5.42	0.29	2.16 to 7.08	4.85	6.00	100.0

N: number; SE: Standard Error; CB: confidence bound; D= day of study; DC: day of challenge; RT-qPCR positive: > 1.7 log₁₀ RNA copies/ml

Viremia and Shedding

Prior to statistical analysis the RT-qPCR data was transformed using an appropriate logarithm transformation. The transformed data was analyzed using a general linear
 1640 repeated measured mixed model. Pairwise treatment comparisons was made at each time point if the treatment or treatment by time point interaction effect was significant ($P \leq 0.05$). Treatment least squares mean and 95% confidence intervals were back-transformed for presentation. Percentage of days viremic/shedding was also be calculated. Each sample was determined to be positive if >50 PRRSV RNA copies/mL, which corresponds to a half of

1645 the detection limit of the technique (100 PRRSV RNA copies/mL). It was also determined if
an animal was ever viremic or ever shed for Days $\leq$ DC (reference to pre-challenge) and
Days $>$ DC (post-challenge).

The objective of the present study was to evaluate the influence of maternally
derived antibodies (MDA) on the efficacy of the EU PRRS MLV, when administered in 1
1650 day-old piglets by the intramuscular (IM, group T02) or the intranasal (IN, group T03) route.
The efficacy was evaluated in seropositive pigs vaccinated at 1 day of age upon inoculation
with a pathogenic genotype I PRRSV isolate as a respiratory challenge. The animals were
challenged at the time when the levels of MDAs detected by SNT in the T01 group became
undetectable (Day 67).

1655 The primary variable in determining the efficacy was viral load in serum (viremia) of
treatment groups T02 and T03 in comparison to T01 pigs. Lung lesions, rectal
temperatures, shedding, clinical signs and body weight were compared as secondary
variables.

The test was valid since all T01 pigs remained PRRSV viro-negative throughout the
1660 vaccination period and no confounding disease factors were detected.

A protective effect of IM vaccination was observed when comparing viral load in
serum between T01 and T02 groups; the group vaccinated with the IVP by the IM route had
significantly lower viral titers compared to the control group T01 at all sampling days post-
challenge. No differences in viral titers were detected between T01 and T03 groups,
1665 indicating no protective effect of IN vaccination on viral load post-challenge. The lack of
efficacy in T03 group was also evidenced by the significant higher viral load observed in this
group compared to the T02 group at 3 and 10 days post-challenge.

The protection conferred following IM vaccination (T02 group) was supported by the
significant reduction in the percentage of nasal shedders as well as in the amount of virus
1670 detected in nasal and oral secretions in the T02 group in relation to the control group T01.
Similar to what was observed for viremia, no positive effect of vaccination was observed in
virus shedding when the IVP was administered by the IN route (T03 group), since no
differences were detected between T01 and T03 groups. In fact, the amount of virus shed
by both the nasal and oral route detected in the T03 group was also significantly higher than
1675 in the T02.

Vaccination by the IM route had also a positive impact on rectal temperatures. Before challenge (D67), mean rectal temperatures were significantly lower in T02 group compared to T01 and T03; however, since none of the pigs from any group had fever ($RT \geq 40.5$) at that time and rectal temperatures at D67 were collected before comingling the animals for the challenge phase, it can be concluded that these differences are probably attributed to stress of the animals that were first evaluated. During the post-challenge period, the percentage of pigs that had fever at least once was 61%, 31%, and 42% in T01, T02 and T03 groups, respectively. Control pigs (T01) had significantly higher rectal temperatures than pigs vaccinated with the IVP (T02 and T03 groups) at day 70 (3 post-challenge); however, at DC+10, rectal temperatures of T03 group were significantly higher than T01 and T02 groups. Besides increased rectal temperatures, no other clinical signs compatible with a PRRSV infection were observed. Regarding body weight, no effect of vaccination was observed as seen by the lack of significant differences between groups.

At necropsy, 13/18 pigs (72%) from the control group T01 had a positive lung visual score. In the T02 and T03 groups, 7/16 (44%) and 13/19 (68%) pigs scored positive as well. Comparison between treatment groups showed no significant differences in the % of lung with lesions. However, the differences observed between the control group T01 and the group vaccinated with the IVP by the IM route (T02) were close to significance ($p=0.092$).

All pigs had presence of PRRSV-specific antibodies before vaccination as measured by ELISA (S/P ratio ≥ 0.4), thus complying with the inclusion criteria. Before challenge (67 days post-vaccination), 39% of the pigs in the control group were still seropositive, indicating the presence of remaining MDAs at that time (mean S/P ratio: 0.279). However, the fact that all pigs from the control group developed viremia after challenge and that 13/18 had also a positive lung score at necropsy indicates that the remaining MDA detected by ELISA did not interfere with the challenge take. In fact, when the levels of PRRSV-specific neutralizing antibodies were determined in those pigs by means of an SNT, negative results were detected in all T01 pigs before challenge (Day 52). In the group vaccinated with IVP by the IM route, 9/16 pigs experienced an increase in the levels of antibodies detected by ELISA from the day of vaccination to 67 days post-vaccination and all of them were seropositive before challenge (mean S/P ratio: 1.803), indicating the development of an antibody response following the IM vaccination even in the face of MDAs. In contrast, only 2/19 pigs vaccinated with the IVP by the IN route had an increase of PRRS antibodies from vaccination to challenge, and only 32% were seropositive at D67 (mean S/P ratio: 0.328), which indicates that no humoral immune response was induced in the other 17 pigs due to neutralization of the vaccine by MDA. After challenge (D77), all pigs, regardless of the

treatment group, were seropositive and no significant differences in ELISA titers were detected between groups.

The EMA/CVMP/WP/439467/2007 guideline "Reflection paper on the demonstration of a possible impact of maternally derived antibodies on vaccine efficacy in young animals" states that the efficacy of a vaccine in animals vaccinated in the presence of MDAs should be, notwithstanding normal biological variation, similar to that obtained in animals of the same age but vaccinated in the absence of MDAs. Based on those specifications, efficacy observed in the present study should be similar to that obtained in a previous study (C/394/13), in which the OOI was evaluated in seronegative animals vaccinated at 1 day of age by the IN and IM routes.

When the EU PRRS MLV was administered by the IM route, efficacy could be demonstrated regardless of the serological status of the pigs, since a significant reduction on viremia (primary variable) was observed in both studies. The protective effect of IM vaccination was also demonstrated for nasal shedding and rectal temperatures in both cases. In addition, following IM vaccination of MDA+ pigs, oral shedding was also significantly reduced and differences in the % of lung with lesions were close to significant. Altogether, these data clearly demonstrate lack of interference of MDA with vaccine efficacy, when the vaccine is administered in 1 day old piglets by the IM route.

When the EU PRRS MLV was administered by the IN route, efficacy could only be demonstrated in the absence of MDA, as seen by the significant reduction in viremia and nasal shedding observed in seronegative animals. In MDA+ pigs, IN vaccination did not induce protection as seen by the lack of significant differences in any of the parameters evaluated.

Thus, vaccination with a single administration of the EU PRRSV MLV vaccine containing a dose of $2.5 \log_{10} \text{CCID}_{50}$ to 1 day-old seropositive pigs by the IM route was protective against PRRSV, as seen by the significant reduction of the viral load detected in serum after challenge with a pathogenic EU PRRS strain 67 days (9.6 weeks) post-vaccination. Efficacy was also supported by the significant reduction of nasal and oral shedding as well as rectal temperatures post-challenge. In addition, differences in mean percentage of lung lesions were close to significance ($p=0.09$). Vaccination with a single administration of the EU PRRSV MLV vaccine containing a dose of $2.5 \log_{10} \text{CCID}_{50}$ to 1 day-old seropositive pigs by the IN route was not protective against PRRSV challenge. The results of the present study demonstrate lack of interference of maternally derived

antibodies with vaccine efficacy, when the vaccine is administered intramuscularly in 1 day old. When the vaccine is administered by the IN route, the majority of the pigs (17/19) failed to generate an immune response following vaccination as measured by ELISA. This indicates neutralization of the vaccine by MDA, which might compromise vaccine efficacy at the herd level.

1750 Example 6- DISSEMINATION, SPREAD AND SAFETY OF THE ADMINISTRATION OF ONE DOSE, REPEATED DOSE AND OVERDOSE OF THE MODIFIED LIVE EU PRRSV VACCINE 96V198 CLONE 1 MSV+3 TO 1 DAY OLD PIGS

1755 The objective of this study was to evaluate the safety of a repeated dose and an overdose, and the dissemination, spread and safety of one dose, of the modified live PRRSV vaccine 96V198 clone 1 administered at 1 day old pigs by IM and IN route. The test was carried out using the MSV+3, which is the least attenuated passage level (see Page 8, MSV+5 is standard Passage 49) that is present in a batch of vaccine.

1760 According to the European legislation in force, it is compulsory to demonstrate the safety of a vaccine to get the marketing authorization. In this case, the protocol was developed taking account European Pharmacopoeia; directive 2009/9/EC and VICH GL44 guidelines to demonstrate the safety of one dose, repeated dose and overdose by IN and IM routes in 1 day old piglets -the proposed routes and category of animals for which the vaccine is intended.

1770 Safety was evaluated in accordance with the European Pharmacopoeia recommendations for vaccines of veterinary use, i.e.: assessment of rectal temperature, and local and general reactions at least until 14 days after inoculation. The spreading of the vaccine strain was evaluated from vaccinated (one dose) to unvaccinated sentinel pigs; shedding (nasal mucus, oral fluids and faeces) and dissemination in tissues in vaccinated animals (one dose) was also investigated.

1775 The study was carried out following the Good Laboratory Practice (GLP) regulations, in accordance with the corresponding OCDE Guidelines and in compliance with the requirements of European Pharmacopoeia 7.7 04/2013:50206 (Evaluation of safety of veterinary vaccines and immunosera).

1780 The test product was EU PRRS MLV (96V198 clone 1), least attenuated passage
level MSV+3), at a maximum release titer of 105.2 TCID₅₀/2 ml with a 2L IN or IM dose,
with the lower passage (less attenuated) being chosen as a better measure of safety. For a
test system, the protocol was developed taking account European Pharmacopoeia; directive
2009/9/EC and VICH GL44 guidelines to demonstrate the safety of one dose, repeated
1785 dose and overdose by IN and IM routes in 1 day old piglets -the proposed category of
animals for which the vaccine is intended.

Sufficient number of sows (n=11) were used to obtain the piglets needed to carry out
the study (at least, n=80). The design applied allowing an appropriate description of the
1790 safety, dissemination and shedding of the vaccine for both routes, IM and IN. Sows arrived
to the experimental farm from a PRRSV negative farm (Annex I). Sows were located in 11
isolated boxes in facilities. At the experimental farm, sows were bled before parturition and
analyzed again for the presence of PRRSV-specific antibodies using a commercially ELISA
(PRRS X3 Ab Test: Ref. 99-4095, IDEXX Laboratories;) (Annex I). IM administration of
1795 Ceftiofur sodium (13ml/sow;; to sows was done as a preventive treatment.

Farrowing of the sows was synchronized administrating 1 ml IM of D-Cloprostenol
(Galapan, INVESA;) (Annex XVI) approximately 111-113 days after insemination (with
synchronization of parturitions). Piglets were born within the next 40 hours (09th and 10th,
1800 April). Start of vaccination was done at 10th; at this date, the age of all piglets was
approximately ≤ 24h (1 day of age).

Cross-fostering was performed within the first day post-farrowing (before
vaccination) to balance litters and to homogenise the litters. Eventually, ten sows with a total
1805 number of piglets in each group equal or higher than the minimum sample size to carry out
safety studies established in the European Pharmacopoeia were used. Total number: 80
piglets; 40 piglets for each route: IN and IM (28 vaccinated with the experimental product –
dose, repeated dose and overdose-, 8 animals as controls, and 4 as sentinels).

1810 Thirty extra-piglets were also distributed in all groups from T01 to T12 as reserve, to
be included in the study only if some group did not reach the minimum number of piglets
needed, due to piglet dead or exclusion (Annex II and Annex XIV; Amendment n°2); raw
data of those piglets were recorded in raw data extra-notebooks marked with an (S)
(notebooks from number 13 to 24). Eventually, ten sows were used and each of them
1815 maintained eleven piglets.

Experimental hypothesis

This study was designed to demonstrate the safety of the vaccine EU PRRS MLV in 1-day-old pigs (96V198 clone 1) under experimental conditions by comparison with a control group inoculated with the vaccine diluent (negative controls) for all parameters described below, but the temperature was analyzed taking into account the basal temperature of each piglet before vaccination as control value. The vaccine was considered safe if no severe local or systemic reactions were observed of causes attributable to the vaccine and the average temperature increase for all pigs in the vaccinated group did not exceed 1,5°C compared to the basal average temperature before vaccination and no piglets in the experimental group showed a temperature rise greater than 2 °C in comparison with each piglet basal temperature before vaccination. Spread and dissemination of the vaccine strain was evaluated investigating the presence of the vaccine strain in sentinel pigs. Dissemination in vaccinated animals was evaluated investigating the quantity of the virus in blood, nasal, oral and rectal samples and in tissues.

Rectal Temperatures- Intranasal and intramuscular routes.

Regarding temperatures, it can be concluded that the vaccine is safe in all the protocols evaluated (route, titer and number of doses) because the results obtained accomplish the European Pharmacopoeia recommendations for vaccines of veterinary use.

Local reactions

Intranasal route. No local reactions were observed.

Intramuscular route.

At necropsy, only two piglets in T12 (maximum titer by IM route) had macroscopic mild lesions. Microscopically, lesions were only observed in four pigs from group T10; lesions were characterized by a very mild focal or multifocal presence of mononuclear inflammatory cell infiltrates.

Systemic reactions and adverse events

Intranasal route: The absence of systemic reactions and clinical signs related to vaccination point out that the vaccine is safe.

Intramuscular route.

Adverse events after the vaccine administration were observed only in 2 out of 8 piglets belonging to the group receiving the highest titer (x10 maximum release titer) by IM

route (group T12). These events disappeared before 4 hours and piglets completely recovered. Regarding the clinical daily evaluation, the cases of lameness cannot be considered as phenomena related to the vaccine administration; lameness was observed also in piglets from control groups. The cause beyond the solely death observed during the study was due to crushing; therefore, it should be considered that this death was not related to the vaccine administration. In summary, the vaccine strain 96V198 CLONE 1 by IM route should be considered as completely safe with the exception of the adverse events described for the highest titer (x10).

Body weight

Intranasal route.

When means of weight gain were compared, T07 showed lower values than the corresponding control (T02) ($p < 0.05$) from day 0 to 14. However, this difference disappeared when weight gains were compared from day 14 to 28 and, especially, when all the study –from day 0 to 28– was taken into account. Therefore, the weight gain of piglets vaccinated by intranasal route was not affected, independently of the dose received.

Intramuscular route.

In the same way, T11 showed lower values than the corresponding control (T04) ($p < 0.05$) from day 0 to 14. This difference disappeared when period from 14 to 28 was analyzed but it appeared again taken all the study –from day 0 to 28–. These differences could be explained because weight gain in three piglets from T11 were the lowest ones including all the piglets necropsied at day 28, independently of the product, titer or route of administration (it means T02, T04, T07 and T11). All the individual weight gains from day 0 to 28 were equal or higher than 5.5 kg, except for the three abovementioned piglets (weight gains 4.08, 2.89 and 3.05 kg for n°199, 202 and 205 piglets, respectively). Interestingly, these piglets suffered lameness for the longest period. In conclusion, it cannot be discriminated if the differences observed between T04 and T11 weight gains were due to the lameness events or to the vaccination.

Dissemination

Intranasal and intramuscular routes. Dissemination of the virus was demonstrated by the presence of vaccine strain in almost all the tissues evaluated in vaccinated animals, independently of the route used. The presence of the strain 96V198 CLONE 1 at titers that could be considered as high in lungs, tonsils and tracheobronchial lymph nodes but also in

1890 spleen and mesenteric lymph nodes suggest a complete organic dissemination of the vaccine strain.

Spread

1895 Intranasal and intramuscular routes. Although the detection of vaccine strain in oral fluid and rectal swabs were inconstant from vaccinated piglets, it was constant and high in nasal swabs; the frequency distribution of vaccine strain detection decreased along time. These results together with detection of vaccine strain in blood samples from all sentinel pigs, demonstrate that 96V198 CLONE 1 was actively and continuously shed early after vaccination. Also, presence of the vaccine strain in tissues and blood samples from sentinel piglets pointed out that the virus spread from vaccinated piglets was active; it could infect 1900 sentinel animals and it could replicate in them; and even one IM sentinel pig from T09 had lung lesions compatible with PRRS plus a positive result for IHC. In conclusion, the above-mentioned results indicate that 96V198 CLONE 1 could be consistently transmitted from vaccinated to non-vaccinated piglets, at least during the early period after vaccination.

1905 Lung Lesions

Intranasal and intramuscular routes. All nine pigs with macro and microscopic lesions compatible with PRRS (interstitial pneumonia) plus a positive result for PRRSV IHC were from IM groups. Thus, it can be concluded that IN route was safe, whereas IM route could induce lung lesions compatible with PRRSV at least in one pig of each group (from 1910 T09 to T12).

Example 7- Safety of the repeated administration of a single dose of a Modified Live EU PRRSV Vaccine when Administered to PRRSV seropositive Pregnant Sows

1915 The present study was designed to demonstrate the safety of the administration of a repeated single dose of a EU PRRS MLV in seropositive sows at second half of pregnancy (87 days of gestation). No systemic reactions post-vaccination were observed following the first or second administration. In addition, no clinical observations were recorded in any sow throughout the whole observation period.

1920 Regarding rectal temperatures, none of the sows showed fever at any point after vaccination ($RT \geq 40.1^\circ\text{C}$). In addition, the mean rectal temperature post-vaccination did not exceed the mean rectal temperature before vaccination plus 1.5°C and none of the sows exceeded its own rectal temperature before vaccination plus 2°C .

Local reactions at the injection point were observed in 63% and 50% of the sows vaccinated with the test item at 1st and 2nd administration, respectively. Reactions consisted of palpable and/or visible swelling (from 0.4 to 2.8 cm of diameter) which disappeared between 1 to 6 days. None of the sows showed reddening, increase in local heat or pain at the injection point following any administration.

Regarding the reproductive performance, no abortions or premature farrowings were recorded. In addition, no apparent effect of vaccination was observed on the number stillborns, mummies, low viable piglets and pre-weaning mortality. Vaccination did not induce transplacental infection as seen by the RT-qPCR negative results detected in serum samples collected at birth and at weaning from the piglets. In conclusion, the repeated administration of the vaccine is safe in seropositive sows in the second half of pregnancy.

Abbreviation used herein include: BHK-21, baby Hamster Kidney clone 21; BRP, Batch Release Protocol; CCID50; Cell Culture Infectious Dose 50%; CP, Control Product; D, Day; DRAC, Daily Review Animal Care; ID, Identification; GLP, Good Laboratory Practices; IF, Immunofluorescence; IV, Intravenous; MLV, Modified Live Vaccine; MSV, Master Seed Virus; NA, Not applicable; P, Passage; PBS, phosphate Buffered Saline; PI, Post-inoculation; PRRSV, Porcine Reproductive and Respiratory Syndrome Virus; RT, Rectal Temperatures; RT-qPCR, Quantitative Reverse-Transcription Polymerase Chain Reaction; SOP, Standard Operating Procedure; S/P, Sample to positive; TBD, To Be Determined; TI, Test Item, and VICH, Veterinary International Conference on Harmonization.

Study Objective and Justification

EU PRRSV modified live vaccine (MLV) is intended for active immunization of gilts and sows in a PRRS contaminated environment, to reduce viremia, transplacental infection and abortion caused by infection with European strains of PRRS virus. According to the legislation in force, it is compulsory to demonstrate the safety of a vaccine to get the marketing authorization. This study was designed in accordance to European Pharmacopoeia 7.7:04/2013/50206 -Evaluation of safety of veterinary vaccines and immunosera- and VICH GL44 -Target animal safety for veterinary live and inactivated vaccines- guidelines. The objective of this study is to evaluate the safety of the repeated administration (1x + 1x) of the EU PRRS MLV in seropositive sows at second half of pregnancy. Material tested was 6.6 log₁₀CCID₅₀ / vial of EU PRRSV, strain 96v198c1, (MSV+3), with a dose of 5.2 log₁₀CCID₅₀ in 2 mL / Intramuscular. RT qPCR

Viremia (PRRSV load in serum) was measured by means of a Reverse Transcription (RT) qPCR performed following standard procedures. In brief, the purified viral RNA was used as template, reverse transcribed at 50°C for 30 minutes, and denatured at 95°C for 5 minutes. The PCR program of reactions consisted of 40 cycles of denaturation at 95°C for 20 seconds and annealing at 53°C for 40 seconds. The qRT-PCR was conducted in a 7500 Real-Time PCR System thermocycler.

The sequences of primers and probe are the following (SEQ ID NOS 6-8, consecutively):

Forward primer (Lelystad F): 5'-GCACCACCTCACCCAGAC-3' (Final concentration 0.5µM).

Reverse primer (Lelystad R): 5'-CAGTTCCTGCGCCTTGAT-3' (Final concentration 0.5µM).

Probe (Lelystad S): 5'-6-FAM- CCTCTGCTTGCAATCGATCCAGAC –TAMRA-3' (Final concentration 0.6µM).

Serology

Sows sera collected pre-vaccination was tested for antibodies to PRRSV using the IDEXX PRRS X3 ELISA, following the manufacturer's instructions. In brief, serum samples were diluted 1:40 in 96-well plate coated with a recombinant PRRSV antigen and incubated 30 minutes at 18-26°C. After washing, an anti-porcine horseradish peroxidase conjugate was added and plates were incubated for another 30 minutes at 18-26°C. Unbound conjugate was washed away and TMB substrate was added to the wells. Color development was measured at 650 nm. S/P values were recorded for each sample. If the S/P ratio was ≥ 0.4 the sample was classified as positive for PRRS antibodies. If the S/P ratio was < 0.4 the sample was classified as negative. ELISA instructions are included in the study file.

Clinical observations

No abnormal clinical observations were recorded for any sow during the post vaccination periods (from D0 to D28).

Rectal temperatures

Mean rectal temperatures (RT) collected pre-vaccination and at the following four days in each group are shown in **Error! Reference source not found.** and **Error! Reference source not found.** Individual data on rectal temperatures are shown in **Error! Reference source not found.**

In order to study the rectal temperatures, two criteria were followed:

a) The mean rectal temperature post-vaccination of the whole group should not exceed the mean rectal temperature pre-vaccination plus 1.5°C.

1990 1st vac: Mean rectal temperature post-vaccination (at either D0+4h, D1, D2, D3 or D4) $\leq$ [Mean Rectal temperature Pre-vaccination (Mean D-1/D0)] + 1.5 °C

2nd vac: Mean rectal temperature post-vaccination (at either D14+4h, D15, D16, D17 or D18) $\leq$ [Mean Rectal temperature Pre-vaccination (Mean D13/D14)] + 1.5 °C

b) The individual post-vaccination rectal temperature of each sow should not exceed the pre-vaccination rectal temperature (Pre-vac) plus 2°C.

1995 1st vac: Individual rectal temperature post-vaccination (at either D0+4h, D1, D2, D3 or D4) $\leq$ [Individual rectal temperature Pre-vaccination (Individual mean D-1/D0)] + 2.0°C

2000 2nd vac: Individual rectal temperature post-vaccination (at either D14+4h, D15, D16, D17 or D18) $\leq$ [Individual rectal temperature Pre-vaccination (Individual mean D13/D14)] + 2.0 °C

2005 In both T01 and T02 groups, the mean RT post-vaccination did not exceed the mean pre-vaccination plus 1.5°C. In addition, the RT post-vaccination of each sow did not exceed the same sow pre-vaccination rectal temperature plus 2°C in any of the sows from T01 or T02 groups. Following the first administration, the maximum increase in RT was 0.81°C (Day 2) in T01 and 0.76°C (Day 0+4h) in T02 group. Following the second administration, the maximum increase was observed at Day 17 in both T01 (0.81°C) and T02 (0.47°C) groups. None of the sows from T01 or T02 group showed elevated rectal temperatures (≥ 40.1 °C) at any time-point after vaccination.

Systemic reactions post-vaccination

2010 No systemic reaction post-vaccination was observed in any of the sows from T01 or T02 group.

Local reactions at injection site

2015 Before vaccination, all sows were inspected for the presence of local reactions in the neck area caused by previous inoculations that might have interfered with the evaluation of local reactions at the injection site. Three sows, two from the T01 group (1802 and 2259)

and one from the T02 (1663) showed old reactions in the right neck due to previous inoculations. However, all these reactions were located below and were remote from the respective inoculation point. When the left neck was inspected before second vaccination, old reactions were also observed in four sows, two from the T01 group (316 and 1802) and two from the T02 group (2547 and 2842). As observed for the right neck, these reactions were located below the inoculation point. In all cases (for both right and left neck), old reactions were not considered to potentially interfere with the evaluation of local reactions post-vaccination. None of the sows vaccinated with the CP (T01 group) showed local reaction at the injection site at any time-point post-vaccination. Local reactions observed post-vaccination in the T02 group are summarized in 17.

Following the first vaccination, 63% of the T02 sows (5/8) showed a visible and/or palpable swelling at the injection site in the right neck. The maximum swelling observed was a reaction of 2.8 cm of diameter (corresponding to a score of 3) that lasted for 6 days. None of the sows showed reddening, increase on local heat or pain at the injection point.

Following the second vaccination, visible and palpable swelling (left neck) was observed in 50% of the T02 sows (4/8). All observed reactions were scored as 1 or 2 (maximum 1 cm of diameter), and lasted a maximum of 5 days. As observed following the 1st vaccination, none of the sows showed reddening, increase on local heat or pain at the injection point.

Table 18. Summary of local reactions in T02 group

Neck side	Visible Reddening	Visible swelling	Swelling score range	Duration range (days)	Palpable swelling	Pain reaction	Increase heat
Right	0/8 (0%)	4/8 (50%)	1 - 3	1 - 6	3/8 (38%)	0/8 (0%)	0/8 (0%)
Left	0/8 (0%)	4/8 (50%)	1 - 2	1 - 5	4/8 (50%)	0/8 (0%)	0/8 (0%)

Postmortem examination of injection sites

At the macroscopic examination, it was observed that several sows from both groups had lesions in the neck muscles, mainly consisting of multiple nodules of caseous material, which were spread in the whole neck area. These lesions were attributed to old injections because they were not detected at the injection point.

When the inoculation points of T02 sows were inspected, 1/8 presented visible reaction on the right neck and 3/8 on the left neck. In the T01 group, one sow (1/7) showed

also visible reaction at the first inoculation point (right neck). All reactions were described as presence of pale muscular fibers, in most cases clearly following the needle trajectory and not measurable. In addition, multiple nodules were observed in two T02 sows (left inoculation point).

Reproductive performance

No abortions or premature parturitions were recorded in any treatment group. Gestation length ranged between 114 and 117 days in the T01 group and 114 and 119 in the T02 group. Piglets born healthy for each sow were identified with ear tags and were maintained with the

In the present study, the safety of the repeated administration of a single dose of the EU PRRS MLV was evaluated at second half of pregnancy (87 days of gestation) in seropositive sows. Safety parameters included systemic reactions to the vaccination, rectal temperature post-vaccination, clinical observations, local reactions at the injection site, reproductive performance and post-mortem examination of injection sites.

No systemic reactions post-vaccination were observed following first or second administration. In addition, no general clinical observations were recorded in any sow throughout the whole study.

Regarding rectal temperatures, none of the sows showed fever at any point after vaccination ($RT \geq 40.1^\circ\text{C}$). In addition, the mean rectal temperature post-vaccination did not exceed the mean rectal temperature before vaccination plus 1.5°C and none of the sows exceeded its own rectal temperature before vaccination plus 2°C .

Local reactions at the injection point were observed in 63% and 50% of the T02 sows at 1st and 2nd vaccination, respectively. Following the 1st vaccination, the maximum swelling observed was a reaction of 2.8 cm of diameter that lasted a maximum of 6 days. Following the second vaccination, the maximum swelling was 1 cm of diameter and the maximum duration was 5 days. None of the sows showed reddening, increase in local heat or pain at the injection point following any administration.

At the postmortem examination, visible reaction at the inoculation point was observed only in 3/8 vaccinated sows. Two of them (15 and 1663) corresponded to the ones with the highest swelling score (2 and 3, respectively) given at the evaluation of local reactions; macroscopically, the lesions were described as pale muscular fibers following the

needle trajectory, which were diagnosed as moderate granulomatous myositis with
2075 involvement of muscular fibers. In the case of sow 15, vaccine drops were observed inside
the lesion, supporting the fact that this lesion was induced as a result of the administration
of the test item. For the other T02 sow (2547) that showed visible reaction, the lesion was
also described as pale muscular fibers following the needle trajectory, but in this case also
2080 with multiple nodules containing caseous material. At the histopathological analysis, this
lesion was diagnosed as severe granulomatous myositis with presence of focal but
extensive necrosis. Since the presence of multiple nodules in the neck area, but not
specifically in the inoculation point, was observed also in 8/8 T02 and in 5/7 T01 sows, it is
possible that the lesion observed in sow 2547 was caused by previous injections rather than
by the test item. This would be supported by the fact that, during the *in vivo* evaluation of
2085 local reactions, this sow had only a swelling of <0.5 cm of diameter that lasted 5 days.

PRRSV-associated reproductive disorders are characterized by increase in
premature farrowings, late term abortions, stillborn or low viable piglets and mummified
foetuses. In the present study, no abortions or premature farrowings were recorded. In
addition, no apparent effect of vaccination was observed on the number stillborns, mummies
2090 or low viable piglets. PRRSV infection might also increase the pre-weaning mortality. In the
present study, the mean % of pre-weaning mortality was 6.0 and 5.3 in T01 and T02 groups,
respectively, indicating no impact of vaccination on piglet survival.

Finally, all serum samples collected at birth or at weaning from piglets born from both
T01 and T02 sows were PRRSV negative by RT-qPCR, indicating the lack of transplacental
2095 infection following vaccination.

Thus, the administration of a repeated single dose of the EU PRRSV MLV to PRRSV
seropositive sows at 87 days of pregnancy: did not induce abnormal systemic reactions
(anaphylactic shock, vomiting); did not induce any clinical observation during the 14 days
post-vaccination; did not induce abnormal rectal temperatures post-vaccination; caused
2100 local reactions at the injection site in 63% and 50% of sows after the 1st and 2nd vaccination,
respectively wherein such reactions consisted of palpable and/or visible swelling (from 0.4
to 2.8 cm of diameter) which disappeared between 1 to 6 days; had no impact on the
reproductive parameters; and did not cause transplacental infection.

Claims

1. A vaccine when used in protecting a porcine animal against infection by a PRRS virus, which vaccine comprises: (a) a European PRRS virus encoded by the polynucleotide molecule of SEQ ID NO: 5 (b) a polynucleotide molecule that is at least 90% identical to SEQ ID NO: 5 comprising mutations compared to SEQ ID NO: 1 selected from:
- in the ORF1a amino acid sequence, S or a conservative substitution thereof at amino acid position 19; Y or a conservative substitution thereof at amino acid position 24; A or a conservative substitution thereof at amino acid position 156; Y or a conservative substitution thereof at amino acid position 157; D or a conservative substitution thereof at amino acid position 268; H or a conservative substitution thereof at amino acid position 294; Y or a conservative substitution thereof at amino acid position 416; S or a conservative substitution thereof at amino acid position 742; L or a conservative substitution thereof at amino acid position 884; P or a conservative substitution thereof at amino acid position 908; K or a conservative substitution thereof at amino acid position 916; K or a conservative substitution thereof at amino acid position 977; S or a conservative substitution thereof at amino acid position 1138; F or a conservative substitution thereof at amino acid position 1160; S or a conservative substitution thereof at amino acid position 1500; R or a conservative substitution thereof at amino acid position 2094; P or a conservative substitution thereof at amino acid position 2254; and L or a conservative substitution thereof at amino acid position 2290;
- in the ORF1b amino acid sequence, S or a conservative substitution thereof at amino acid position 567; and H or a conservative substitution thereof at amino acid position 912;
- in the ORF2a amino acid sequence, L or a conservative substitution thereof at amino acid position 22; F or a conservative substitution thereof at amino acid position 88; M or a conservative substitution thereof at amino acid position 94; and F or a conservative substitution thereof at amino acid position 95;
- in the ORF2b amino acid sequence, L or a conservative substitution thereof at amino acid position 47;
- in the ORF3 amino acid sequence, S or a conservative substitution thereof at amino acid position 52;

in the ORF4 amino acid sequence, T or a conservative substitution thereof at amino acid position 151;

in the ORF5 amino acid sequence, F or a conservative substitution thereof at amino acid position 20; and D or a conservative substitution thereof at amino acid position 37;

in the ORF5a amino acid sequence, V or a conservative substitution thereof at amino acid position 18; and R or a conservative substitution thereof at amino acid position 35; (c) said encoding polynucleotide molecule; (d) said polynucleotide molecule in the form of a plasmid; or (e) a viral vector comprising said polynucleotide molecule;

in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use, and wherein said use of the vaccine provides early and safe vaccination of piglets prior to weaning including from one day old or less to three weeks of age, and wherein said use of the vaccine provides a duration of immunity for up to 6 months.

2. The vaccine when used according to Claim 1, wherein said vaccine provides protection against infection when administered to a piglet that is about 8-12 hours old.
3. The vaccine when used according to Claim 1 wherein onset of immunity is provided beginning at two weeks after vaccination.
4. The vaccine when used according to Claim 1 wherein onset of immunity is provided beginning at 24-28 days after vaccination.
5. The vaccine when used according to Claim 1, wherein said vaccine comprises a European PRRS virus encoded by the polynucleotide molecule of SEQ ID NO: 5.
6. Use of a vaccine for the manufacture of a medicament for protecting a porcine animal against infection by a PRRS virus, which vaccine comprises: (a) a European PRRS virus encoded by the polynucleotide molecule of SEQ ID NO: 5 (b) a polynucleotide molecule that is at least 90% identical to SEQ ID NO: 5 comprising mutations compared to SEQ ID NO: 1 selected from:

in the ORF1a amino acid sequence, S or a conservative substitution thereof at amino acid position 19; Y or a conservative substitution thereof at amino acid position 24; A or a conservative substitution thereof at amino acid position 156; Y or a conservative substitution thereof at amino acid position 157; D or a conservative

substitution thereof at amino acid position 268; H or a conservative substitution thereof at amino acid position 294; Y or a conservative substitution thereof at amino acid position 416; S or a conservative substitution thereof at amino acid position 742; L or a conservative substitution thereof at amino acid position 884; P or a conservative substitution thereof at amino acid position 908; K or a conservative substitution thereof at amino acid position 916; K or a conservative substitution thereof at amino acid position 977; S or a conservative substitution thereof at amino acid position 1138; F or a conservative substitution thereof at amino acid position 1160; S or a conservative substitution thereof at amino acid position 1500; R or a conservative substitution thereof at amino acid position 2094; P or a conservative substitution thereof at amino acid position 2254; and L or a conservative substitution thereof at amino acid position 2290;

in the ORF1b amino acid sequence, S or a conservative substitution thereof at amino acid position 567; and H or a conservative substitution thereof at amino acid position 912;

in the ORF2a amino acid sequence, L or a conservative substitution thereof at amino acid position 22; F or a conservative substitution thereof at amino acid position 88; M or a conservative substitution thereof at amino acid position 94; and F or a conservative substitution thereof at amino acid position 95;

in the ORF2b amino acid sequence, L or a conservative substitution thereof at amino acid position 47;

in the ORF3 amino acid sequence, S or a conservative substitution thereof at amino acid position 52;

in the ORF4 amino acid sequence, T or a conservative substitution thereof at amino acid position 151;

in the ORF5 amino acid sequence, F or a conservative substitution thereof at amino acid position 20; and D or a conservative substitution thereof at amino acid position 37;

in the ORF5a amino acid sequence, V or a conservative substitution thereof at amino acid position 18; and R or a conservative substitution thereof at amino acid position 35; (c) said encoding polynucleotide molecule; (d) said polynucleotide

molecule in the form of a plasmid; or (e) a viral vector comprising said polynucleotide molecule;

wherein said medicament is to be used in an amount effective to produce immunoprotection against infection, and a carrier suitable for veterinary use, and wherein said use of the vaccine provides early and safe vaccination of piglets prior to weaning including from one day old or less to three weeks of age, and wherein said use of the vaccine provides a duration of immunity for up to 6 months.

1/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

ATGATGTGTAGGGTATTCCCCCTACATACACGACACTTCTAGTGTTTGTG
TGCCTTGGAGGCGTGGGTATAGCCCCGCCCCACCTCTTGGCCCCCTGTTCT
AGCCCAACAGGTATCCTTCTCTCTCGGGGCGAGCGCGCCGCCTGCTGCTC
CCTTGCAGCAGGAAGGACCTCCCGAGTATTTCCGGAGAGCACCTGCTTTA
CGGGATCTCCACCCTTTAACCATGTCTGGGACGTTCTCCCGGTGCATGTG
CACCCCGGCTGCCCCGGGTATTTTGGAGCGCCGGCCAAGTCTATTGCACAC
GGTGTCTCAGTGCACGGTCTCTTCTCTCTCCGGAGCTTCAGGACACTGAC
CTCGCTGCAATTGGCTTGTTTTACAAGCCTAAAGACAAGCTTCACTGGAA
AGTCCCTATCGGCATCCCTCAGGTGGAGTGTACTCCGTCCGGGTGCTGTT
GGCTCTCAGCCATCTTCCCCTTGGCGCGCATGACCTCCGGCAATCACAAC
TTTCTCCAACGACTTGTGAAGGTTGCTGATGTTTTGTACCGTGACGGCTG
CTTGGCACCTCGACACCTTCGCGAACTCCAAGTTTACGAGCGCGGCTGCA
ACTGGTACCCGATCACGGGGCCCGTGCCCCGGGATGGGTTTGTACGCGAAC
TCCATGCACGTGTCCGACCAACCGTTCCCTGGTGCCGCTTATGTGTTAAC
GAACTCACCTCTGCCTCAGCAGGCTTGTCGACAGCCGTTTTGTCCATTG
AGGAGGTTTCATTCCGACGTATACAGGTGGAAGAAATTCGTGATTTTTGTG
GATTCCTCTTCTGACGGCCGATCTCGCATGATGTGGACACCAGGATCCGA
CGACTCGGCTGCCTTAGAAGTATTGCCACCTGAACTAGAATGTGAGTCG
AAATCCTCGTTTCGGAGTTTTCTTGCCACCAACCCTGTGACATCACCAAC
TGGGAGCTCACTGAGTCCCCTGAGCACGGTTTTTCTTTCAGCACGTCTCA
TTCTTGTGGTTACCTTGCCCAAGACCCTGACGTGTTTGATGGTAAGTGTT
GGCTTTCTTGCTTTTTTGGGCCTGCCGACCGAAGTGTGGCGTCATGAGGAG
CATCTAGCTAACGCCTTCGGTTACCAAACCAAGTGGGGCGTGCATGGTAA
GTACCTCCAGCGCAGGCTTCAGGTCCGCGGCATGCGTGCTGTAGTTGATC
CTGATGGTCCCATCCACGTTGAAGCGCTGTCTTGCCCCCAGTCTTGGATC
AGGCACCTGACTCTAAATGATGACGTCACCCCAGGATTTGTTTCGCCTAAC
ATCCCTTCGCATTGAGCCGAACACAGAGCCTACTACTTTCCGGATCTTTC
GGTTTGGAGCGCATAAGTGGTATGGCGCTGCCGGCAAACGGGCTCGTGCT
AAGCGTGCCGCTAAAGGTGAGAAGAATTCAGCTCCCACCCCCAAGGTCGC
CCTGCCGGTCCCCACCTATGGAATTACCACCTACTCTCCACCGACAGACG
GGTCTTGTGGTTGGCATGTCTTGCCGCCATAATGAACCGGATGATAAAT
GGTGACTTCACGTCCCCTCTGACTCCGTACAACAGACCAGAGGATGATTG
GGCTTCTGATTATGATCTTGCTCAGGCAATCCAATGTCTACAACCTGCCTG

FIG. 1A

2/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

CTACCGTGGTCCGGAGTCGCGCCTGTCCCAACGCCAAGTACCTCATAAAA
CTCAACGGAGTCCACTGGGAGGTAGAGGTGAGGTCAGGAATGGCTCCTCG
CTCTCTTTCTCGTGAATGTGTGGTTGGCGTTTGCTCTGAAGGTTGTGTTG
CACCGCCTTATCCAGCAGACGGGCTACCAGAACGAGCACTCGAGGCCTTG
GCGTCTGCTTACAGGTTACCTCTGATTGTGTTAGCTCTGGCATTGCTGA
CTTTCTTGCTAACCCACCTCCTCAGGAATTCTGGACCCTCGACAAAATGT
TGACCTCCCCGTCACCAGAGCGGTCCGGCTTCTCTAGTTTGTATAAATTA
CTATTAGAGGTTGTTCCGCAAAAATGCGGTGCCACGGAAGGGGCTTTCGT
CTATGCTGTTGAGAGAATGTTGAAGGATTGTCCGAGCTCCAAACAGGCCA
TGGCCCTTCTGGCAAAAATTAAAGTTCCATCCTCAAAGGCCCCGCTCTGTG
TCCCTTGACGAGTGTTTCCCTACGGATGTTTCAGCCGACTTCGAGCCAGC
ATCTCAGGAAAGGCCCCAAAGTTCCGGTGCTGCTGTTGTCCTGTGTTTAC
CGGAAGTAAAAGAGTTTCAAGAAGCAGCCCCAGAAGAAGTTCAAGAGAGT
GGCCATAAGGTCGCCCCTCTGCATTTGTTGCCGAGGGTCCTAACAATGA
ACAGGTACCGATGGCTGCCGGCGAGCAACTGAAGCCCGGTGGTCGCGTTT
TGCGGGTCGGGAATGCTCATGAAGGTGTTCTGGCCTCAACTAGTTTCGACC
AACCTGATAGGCGGGAACCTCCCCCTTCAGACTCCATGAAAGAGAGCAT
GCTCAATAGCTGGGAAGACGAACCACTGGATTTGTCCCAACCGGCACCAG
CTGTTACAATGACCCTTGTGAGAGAGCAAACACCCGACAACCTGGGTCCT
GATGCCGGTGCTTCCCCGTCACCGTTTCGAAAATTTGTCCCGACAGGGCC
TAACTCCGTCATGTTGAGCACTGCGGCACGGAGTCAGGCGACAGCAGTT
CGCCCTTGATCTGTCTTATGCGCAAACCTGGACCAGCCTTTAAATCTA
TCCTTGCCCGCTTGCCAGTGAGGGCCACCGCGTCTGACCCTGGCTGGGT
CCACGGTAGGCGTGAGCCTATCTTTGTGAAGCCTCGAGATGCTTTCTCTG
ACGGCGATTACGCCCTTCAGCTCGGGGAGCTGTCTGAATCCGGCTCCGTC
ATCGAGTTTGACCGGACAAGAAATGCTCCGGCGGTTCGACGCCCTGTTGA
CTTGACGGCTTCGAACAAGGCCCTCTCTGTGGTTGATCCTTTTCAATTTG
CCGAACCTCAAGCGCCCGCGTTTTTCCGCACAAGCCTTAATTGACCGAGGC
GGTCCACTTGCCGACGTCCATGCAAAAATAAAGAACCGGGTATATGAACA
GTGCCTCCAGGCTTGTGAGCCTGGCAGTCGCGCAACCCAGCCACCAAGA
AGTGGCTCGATAAAATGTGGGATAGGGTGGACATGAAGACTTGGCGCTGT
ACCTCGCAGTTCCAAGCTGGTCGCATTCTTGCGTCCCTTAAATTCTCTCC

FIG. 1B

3/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

TGACATGATTCAGGACACACCACCTCCTGTTCTAGGAAGAATCGGGCTA
GTGATAATGCCGGCCTGAAGCGACTGGTGGCGCAGTGGGACAGAAAATTG
AGTGCAACCCCCCTTCAAACCGGTTGGACCAACACTTGACCAAATTGT
CCCTTCGCCCACGGGTACCCAGCAAGAAGATGTCACCTCCTCCGATGGGC
CATCTCATGCGCCGGATCCTCCTAGTCGAATGAGCACGAGTGGGAGTTGG
AAGGGCCTTGTGCTCTCTGGTACCCGTCTCGCGGGGTCCATTAGTCAGCA
TTTCATGACATGGGTTTTTGTAGGTTTTCTCCCATCTCCAGCTTTTGCAC
TCACACTTTTTCTCGCCGAGGGGCTCTATGGCTTCAGGTGATTGGATGTTT
GCAGGTGTTGTTTTACTTGCTCTCCTGCTCTGTCGTTCTTACCCAGTATT
CGGGTGCTTCCCTTATTGGGTGTCCTTTCTGGCTCTGTGCGGCGCGTTC
GTCTGGGGGTTTTTGGTTCTTGGATGGCTTTCGCTGTATTTTTATTCTCG
ACTCCATCCAACCCAGTCGGTTCTTCTTGTGACCACGATTCGCCGGAGTG
TCATGCTGAGCTTTTGGCTCTTGAGCAGCGCCAACCTTGGGAACCTGTGC
GCGGCCTTGTGGTCGGCCCCCTCGGGCCTCTTATGTGTCACTTCTGGCAAG
CTACTCGGTGGGTCACGTTATCTCTGGCATGTTTTCTTACGTTTATGCAT
GCTTGCAGATTTGGCCCTTTCTCTTGTGTTTATGTGGTGTCCCAGGGGCGTT
GTCACAAGTGTGTTGGGGAAAGTGTATAAGAACAGCTCCGGCGGAGGTGGCT
CTCAATGTGTTCCTTTCTCGCGCGCTACCCGTAGCTCTCTTGTGTCTTT
GTGCGATCGGTTCCAAGCGCCAAAAGGGGTTGATCCTGTGTACTTGGCAA
CGGGTTGGCGCGGTGTTGGTGTGGTGAGAGTCCCATTCAACAACCGCAC
CAAAAACCCATAGCTTATGCTAATCTGGATGAAAAGAAAATATCTGCCCA
AACGGTGGTTGCTGTCCACACGATCCCAGTCAGGCCATCAAGTGCCTGA
AAGTTTTGCAGGCGGGAGGAGCCATCGTGGACCAGCCACACCTGAGGTC
GTCCGTGTATCCGAAATCCCCTTCTCAGCCCCATTTTTCCCAAAGTTCC
AGTCAACCCAGATTGTAAGGTGTGGTGGATTCCGACACTTTTGTGGCTG
CGGTTTCGCTGCGGCTACTCGACAGCACAACTGGTCTTAGGCCAGGGCAAC
TTTGCCAAGTTAAATCAGATTCCCCTCAGGAGCTCTATCTCCACCAAAGC
GATTGGCGGGGCTCTTACACCCTTGCTGTGGCTCAAGTTTCTGTGTGGA
CTCTTGTTCACTTCATCCTCGGTCTTTGGTTCACGTCACCCCAAGTGTGT
GGCCGAGGAACCTCTGACTCATGGTGTTCAAATCCTTTTTTCATACCCTAC
CTATGGCCCCGGGGTTGTGTGCTCCTCTCGACTTTGTGTGTCTGCCGACG
GGGTCACCTCTACCATTGTTCTCAGCCGTGGCTCAACTCTCCGGTAGAGAG

FIG. 1C

4/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

GTGGGGATTTTTATTTTGGTACTCGTCTCCTTGATGGCTTTGGCCCACCG
CATGGCCCTTAAGGCAGACATGTTGATGGTCTTTCTGGCTTTTTGTGCTT
ACGCCTGGCCCATGAGCTCCTGGTTGATTTGCTTCTTTCCTACACTCTTG
AAGTGGGTTACCCTCCACCCTCTTACTATGCTTTGGGTGCACTCATTCTT
GGTGTTCCTGCTGCCAGCAGCCGGCATCCTCTCACTAGGGATAACTGGCC
TTCTTTGGGCGGTTGGCCGCTTTACTCAGGTTGCCGGAATTATTACACCT
TATGACATCCACCAGTACACCTCTGGGCCACGTGGCGCTGCAGCTGTGGC
CACAGCCCCAGAAGGCACTTATATGGCCGCCGTCCGGAGAGCTGCTTTAA
CTGGGCGAACTTTAATCTTCACCCCGTCTGCAGTTGGATCCCTTCTCGAA
GGAGCTTTCAGGACTCATAAACCTTGTCTTAACACCGTAAATGTTGTAGG
CTCTTCCCTTGTTCCGGCGGGGTTTTCACTATTGACGGCAGAAAAATTG
TTGTCACTGCTGCCCATGTGTTGAACGGCGACACAGCTAGAGTCACCGGC
GACTCCTACAACCGCATGCATACTTTCAAGACCAATGGTGATTATGCCTG
GTCCCATGCTGATGACTGGCGGGGCGTTGCCCTGCGGTCAAGGTTGCGA
AGGGGTACCGCGGTCTGTGCCTACTGGCAAACATCAACTGGCGTCGAACCC
GGTGTATTGGGGAAGGGTTCGCCTTCTGTTTCACCACCTGTGGCGATTCT
GGGGTCACCCGTCATCTCAGAATCCGGTGATCTCATTGGAATCCATACCG
GTTCAAATAAACTTGTTCTGGTCTTGTGACAACCCCTGAAGGGGAGACA
TGTACCATCAAAGAAACCAAGCTCTCTGACCTCTCCAGACATTTTCGCAGG
CCCAAGCGTTCCTCTTGGGGATATTAAATTGAGTCCAGCCATCATCCCTG
ATATAACATCCATTCCGAGTGACTTGGCATCGCTCCTATCCTCCGTCCCT
GTAGTGGAAGGCGGCCTCTCGACCGTTCAACTTTTGTGTGTCTTTTTCTT
ACTTTGGCGCATGATGGGCCATGCCTGGACTCCCATTGTTGCCGTGGGTT
TCTTTTTACTGAATGAAATCTTCCAGCAGTTTTGGTCCGAGCCGTGTTT
TCTTTTGCACTTTTTGTGCTTGCATGGGCCACCCCTGGTCTGCACAGGT
GTTGATGATTAGACTCCTCACGGCATCTCTCAACCGCAATAAGCTTTCTC
TGGCGTTCTACGCACTCGGGGGGGTTCGTGCGTTTGGCCGCTGAAATCGGG
ACTTTTGCTGGCAAATTGCCTGAATTGTCTCAAACCCTTTCGACATACTG
CTTCTTACCTAGGGTCCTTGCTATGACCAGTTGTGTTCCACCATCATCA
TTGGTGGACTCCATGCCCTCGGTGTAATTCTGTGGTTGTTCAAATACCGG
TGCCTCCACAACATGCTCGTTGGTGATGGGAGTTTTTCAAGCGCCTTCTT

FIG. 1D

5/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

CCTACGGTATTTTGCAGAGGGTAATCTCAGAAAAGGTGTTTCGCAGTCCT
GTGGCATGAATAACGAGTCCCTGACGGCTGCTTTGGCTTGCAAGTTGTCA
CGGGCTGACCTTGATTTTTTGTCCAGCTTAACGAACTTCAAGTGCTTTGT
ATCTGCTTCGAATATGAAAAATGCTGCCGGCCAGTACATTGAAGCGGCGT
ATGCCAAGGCCCTGCGCCAAGAGTTGGCGTCTCTAGTTCAGATTGACAAA
ATGAAAGGAGTTTTGTCCAACTCGAGGCCTTTGCTGAAACAGCTACTCC
GTCCCTTGACATAGGTGACGTGATTGTTCTGCTTGGGCAACATCCACACG
GATCTATCCTTGATATTAATGTGGGGACTGAGAGGAAAACTGTGTCCGTG
CAAGAGACCCGGAGCCTAGGCGGTTCCAAATTCAGTGTTTGTACTGTCGT
GTCTAACACACCCGTGGACGCCTTAACCGGCATCCCACTCCAGACACCAA
CCCCTCTTTTTGAGAATGGTCCGCGTCATCGCAGCGAAGAAGACGATCTT
AAAGTCGAGAGGATGAAGAAACACTGTGTGCCCTCGGCTTCCACAACAT
CAATGGCAAAGTTTACTGCAAGATTTGGGACAAGTCCACCGGTGACACCT
TTTACACGGATGATTCCCGGTATACCCACGACCATGCTCTTCAGGACAGG
TCAGCCGACTACAGAGACAGGGACTATGAGGGTGTGCAAACCGCCCCCA
ACAGGGATTTGATCCAAAGTCTGAAACCCCTGTGCGCACTGTTGTGATCG
GCGGTATTACGTATAACAGGTATCTGACTAAGGGTAAGGAGGTTCTGGTT
CCCAAGCCCGACAATTGCCTTGAAGCTGCCAAGCTGTCTCTTGAGCAAGC
TCTCGCTGGGATGGGCCAAACTTGCGACCTTACAGCTGCCGAGGTGGAAA
AGTTAAAGCGCATCATCAGTCAACTCCAAGGTTTGACCACTGAACAGGCT
TTAAACTGCTAGCCGCTAGCGGCTTGACCCGCTGTGGCCGCGGCGGCTTA
GTTGTTACTGAAACGGCGGTAAAAATTGTAAGATACCACAGCAGAACTTT
CACCTTGGGCCCTTTGGACCTAAAAGTCGCTTCTGAGGTGGAGGTGAAGA
GATCAACTGAGCAGGGCCACGCTGTTGTGGCAAACCTATGTTCTGGTGTT
GTATTGATGAGACCTCACCCACCGTCCCTTGTTGACGTTCTTCTGAAACC
CGGACTTGACACAACACCCGGCATTCAACCGGGGCATGGGGCCGGGAATA
TGGGCGTGACGGTTCCATTTGGGATTTTGAAACCGCACCCACAAAGGCA
GAGCTCGAGTTGTCCAGGCAAATAATCCAAGCATGTGAAGTCAGGCGCGG
GGATGCCCCGAACCTCCAACCTCCCTTACAAGCTCTGTCCAGTTAGGGGGG
ATCCTGAGCGGCATAAAGGCCGCTTATCAATACCAGGTTTGGAGATTG
CCTTACAAGACTCCTCAAGACACCAAGTCCGCAATCCACGCGGCTTGTTG
CCTGCACCCCAACGGGGCTCCCGTGTCTGATGGTAAATCCACATTAGGCA

FIG. 1E

6/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

CCACTCTTCAACACGGTTTTCGAGCTTTATGTCCCTACTGTGCCCTATAGT
GTCATGGAGTACCTTGATTACGCCCTGACACCCCCTTTATGTGTACCAA
ACATGGTACCCCCGAGGCTGCTGCAGAGGACCTTCGAAAATATGACTTAT
CCACCCAAGGGTTTGTCTGCTGGGGTCCTACGTCTAGTACGCAGATTC
ATCTTTGGCCATATTGGTAAGGCACCGCCACTGTTCCCTCCCATCAACTTA
TCCCGCCAAGAACTCTATGGCAGGGATCAATGGCCAGAGGTTCCCAACAA
AAGACGTTTCAGAGCATACCTGAAATTGATGAAATGTGTGCCCGCGCTGTC
AAAGAGAATTGGCAAACCTGTGACGCCTTGCACCCTCAAAAAACAGTATTG
TTCCAAGCCTAAAACCAGGACCATCCTTGGTACCAACAACCTTCATTGCCT
TGGCTCACAGGTCGGCACTCAGCGGTGTCACTCAGGCGTTCATGAAAAAA
GCCTGGAAGTCCCCGATCGCCTTGGGGAAAAACAAATTTAAGGAGTTGCA
CTGCACTGTGCGCGGTAGGTGCCTCGAGGCCGACTTGGCCTCTTGTGATC
GCAGCACCCCGGCCATCGTGAGGTGGTTTGTGCGCAACCTCCTGTACGAA
CTTGCGGGTTGTGAAGAGTACTTGCCAAGCTATGTGCTCAATTGTTGCCA
TGACCTGGTGGAACGCAAAATGGCGCCTTCACAAAACGTGGTGCCCTGT
CATCTGGAGACCCCGTTACCAAGTGTGTCCAACACAGTGTATTCACTGGTG
ATTTATGCTCAGCACATGGTGCTGTGCGCTTTGAAGATGGGTGCATGAAAT
CGGCCTCAAGTTCCTCGAGGAACAGCTTAAGTTCGAGGACCTTCTCGAAA
TCCAGCCTATGTTGGTATATTCTGATGACCTTGTATTGTATGCTGAAAGA
CCCACCTTCCCCAATTACCATTTGGTGGGTGAGCACCTTGACCTGATGCT
GGGTTTTAAGACAGACCCAAAAAAGACTGTCATAACTGACAAACCCAGCT
TTCTTGGCTGTAGAATCGAAGCGGGGCGACAACCTGGTTCCCAGTCGCGAC
CGCATTCTGGCTGCTCTTGCAATACATGAAGGCGCAGAACGCCTCAGA
GTATTATGCGTCTGCTGCCGCGATTCTGATGGACTCGTGTGCTTGCATTG
ACCACGATCCTGAGTGGTATGAGGACCTCATCTGCGGCATTGCTCGATGC
GCCCCCAGGATGGCTACAGTTTCCCAGGCCCGCCGTTCTTCATGTCCAT
GTGGGAGAAGCTGAAAAGTCACAATGAAGGCAAGAAATTCGCCCACTGTG
GCATTTGTGACGCCAAGGCCGATCATGCGTCCGCCTGTGGGCTTGATTTA
TGTCTGTTCCACTCGCACTTTTCATCAACACTGCCCTGTTACTCTGAGCTG
CGGCCATCATGCCGTTTCAAAGAATGCCACAGTGTGAGTCACCTGTTG
GAGCTGGTAAATCCCCCCTTGATGCAGTGTGAAACAAATCCCGTACAAA
CCCCCTCGTCCTGTCATCATGAAGGTGGACAATAAAACAACGACCCTTGA

FIG. 1F

7/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

CCCGGGAAGGTATCAGTCCCGTCGAGGTCTTGTGCTGTCAAGAGGGGTA
TTGCAGGCAATGAGGTTGATCTCGCTGATGGAGACTATCAGGTGGTGCCC
CTTTTGCCAACTGCAAAGACATAAACATGGTGAAGGTGGCTGTCAATGT
GCTACTCAGCAAGTTCATAGTGGGGCCGCCAGGTTCCGGCAAGACGACCT
GGCTACTGAGTCAAGTTCAGGATGATGATGTCATCTATACACCTACCCAT
CAGACCATGTTCGACATAGTCAATGCCCTCAAAGTCTGTAGGTATTCCAT
CCCAGTGGCTTCAGGGCTCCCTTTCCACCGCCCGCCagatctggaCCGT
GGGTTAGGCTCGTTGCTAGCGGGCACATCCCTGGCCGAATATCATACTT
GACGAGGCCGGATATTGCAATCATCTGGATATTCTCAGACTGCTTTCTAA
GACACCTCTTGTGTGTTTGGGTGACCTTCAGCAACTTCACCCTGTGGCT
TTGACTCTTACTGTTATGTTTTTGATCACATGCCTCATAAGCAGCTGACC
ACTATTTATAGATTTGGCCCCAATATCTGTTCTGCCATTCAACCTTGTTA
CAGGGAAAACTCGAATCTAAGGCCAGGAACACTAGGGTGGTTTTTACTG
CCCGGCCCCGTGGCTTTTGGTCAGGTGTTGACGCCATATCATAAAGACCGC
ACCGGCTCAGCTATAACCATAGATTTCATCCCAGGGAGCCACCTTTGATGT
TGTGACGCTGCATTTGCCGTCGCCAGATTCCCTGAACAAATCCCGGGCTC
TTGTAGCTATCACTCGGGCAAGGCATGGGTGTTTCATCTATGACCCTCAT
AACCAACTCCGGAAGTTTTTCAACTTAACACCTGAGCGCACTGATTGCAA
CCTCGTGTTCAACCGCGGGGACGAGTTGGTAGTTTTGAATGCAGATAATG
CAGTCACGACTGTGGCTAAGGTTCTGGAGGCGGGCCCGTCTCGGTTTCGA
GTATCAGATCCAAGGTGCAAGTCTCTTTTAGCCGCTTGCTCGGCCAGTCT
GGAAGGAGGCTGCATGCCACTGCCGCAAGTGGCACATAATCTGGGGTTTT
ACTTCTCTCCAGATAGTCCAGCATTTGCACCTCTGCCAAAAGAGCTGGCA
CCACATTGGCCGGTTGTTACTAGTCAGAACAACCAGGCATGGCCCGACCG
ACTTGTGCTAGTATGCGGCCAGTTGATGCCCCGTACAGCAAGCCTATGG
TCGGTGCAGGGTATGTGGTTGGGCCATCCACTTTCCTTGGCACTCCTGGT
GTGGTGTCACTATCTCACGCTGTACATCAGGGGTGAGCCCCAGGCCTT
GCCGGAGACACTCGTCTCAACGGGACGTATAGCTACTGATTGTGAGAGT
ATCTCGACGCAGCTGAGGAAGAAGCAGCTAAAGAACTCCCTCACGCATTC
ATTGGCGATGTCAAAGGTACTACAGTGGGGGGGTGTCACCACATTACGTC
AAAATACCTTCCCAGGTCCCTTGCCCAAGGACTCCGTTGCCGTGGTGGGAG
TGAGTTCGCCCCGGCAAGGCTGCCAAGCCGTGTGTACTCTCACCGATGTG

FIG. 1G

8/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

TATCTTCCCGAGCTCCGGCCATATTTGCAACCGGAAACAGCATCGAAATG
CTGGAAACTTAACTAGACTTCAGGGATGTCAGACTGATGGTCTGGAAAG
GAGCCACCGCATATTTCCAACCTGGAGGGGCTCACATGGTCAGCGCTGCCC
GACTATGCCAGGTTCAATTCAGCTACCAAAAAGATGCCGTTGTGTACATTGA
TCCGTGCATAGGACCGGCAACAGCCAACCGTAAGGTTGTGAGAACTACAG
ATTGGCGAGCTGACCTGGCAGTGACACCGTACGACTACGGTGCTCAGAGC
ATTTTGACTACAGCCTGGTTCGAGGACCTTGGGCCGCACTGGAAGATTTT
AGGGTTGCAACCCTTCAAGCGGGCATTGTTGGCTTTGAAAACACTGAGGATT
GGCGATCCTTGACGTCGCATGAACGACGGCAAGGACTACACTGACTAT
AACTGGAATTGTGTTGACAACGCCACATGCCATCTACGGACGTGCCCCG
TGACCATACGTATCACTTTGCCCTGGCACTGAACTGCAAGTAGAGCTAG
GTAAACCTCGGCTACCGCCTGAGCAAGTACCGTGAATCTAGAGTGATGCA
ATGGGGTCACTGTGGAGTAAAATCAGTCAACTGTTTCGTGGATGCCTTCAC
TGAGTTCCTTGTTAGTGTGGTTGATATTGTCATCTTCCTTGCCATACTGT
TTGGGTTACCGTTGCAGGGTGGTTACTGGTCTTTTTTCTCAGAGTGGTT
TGCTCCGCGCTTCTCCGTTGCGCTCTGCCATTCACTCTTCCGAACATATC
GAAGGTCCTATGAGGGCTTGCTACCTAATTGCAGACCGGATGTTCCACAA
TTCGCATTCAAGCACCCATTGGGTATGTTTTGGCACATGCGAGTTTCCCA
CTTAATTGACGAAATGGTCTCTCGCCGCATTTATCAGACCATGGAACATT
CAGGTCAAGCGGCCTGGAAGCAGGTGGTTAGTGAAGCTACTCTCACAAGA
CTGTCAAAGCTCGACATAGTTCTCCACTTCCAACACCTGGCCGCAATAGA
GGCGGATTCTTGCCGCTTCCTCAGCTCACGACTTGTGATGCTGAAAAATC
TTGCTGTTGGCAATATGAGCCTACAGTACAACACCACGTTGGACCGCGTT
GAGCTCATCTTCCCAACACCAGGTACGAGGCCCAAATTGACCGATTTTAG
ACAATGGCTCGTCAGTGTGCACGCTTCTATTTTTTCTCTGTGGCCTCAT
CAGTTACCTTGTTCATAGTGCTTTGGCTTCGAATTCCAGCTCTACGCTAT
GTTTTTGGTTTCCATTGGCCCACGGCAACACATCATTGAGCTAACCATC
AATTACTCTATATGTAAGCCCTGTCTCACCAGTCAAGCGGCTCGACAAAG
GCTCGAACCCGGTCGCAACATGTGGTGCAAAATAGGGCACACCACGTGTG
AGGAGCGTGACCATGATGAGTTGTCAATGACCATTCCGTCCGGGTACGAT
AACCTCAAACCTGAGGGTTATTACGCTTGGCTGGCTTTTTTGTCTTTTC
TTACGCAGCCCAATTTTCATCCAGAGTTGTTTCGGAATAGGGAATGTGTCAC

FIG. 1H

9/10

complete genome sequence for SEQ ID NO:5

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

GCGTCTTCGTGGATAAACGGCACCAGTTCATCTGTGCCGAGCACGACGGA
CAAAATTCAACCGTATCCACCGAACATAATTTCCGCATTGTATGCGGC
GTACTACCACCACCAGGTAGACGGGGGCAATTGGTTCCACCTGGAATGGC
TGCGGCCTTTCTTTTCCTCCTGGCTAGTACTCAATATTTTCATGGTTTCTG
AGGCGTTTCGCTGCAAGCCCTGTTTCTCGACGCATTTATCAGATATTGAG
ACCAACACGACCGCGGCTGCCGTTTTCATGGTCCTTCAGGACATCAACTG
TTTCCACAGTGGCTCAGAGGCACAAACGACTGGTCCCATCAGAAAGTCGT
CCCAATGCCGTGAAGCCGTCGGCACTCCCCAGTACATCAAGATAACGGCC
AATCTGACCGACGAATCATACTTGTACAACGCGGACTTGCTGATGCTTTC
TGCGTGCCCTTTCTACGCCTCAGAGATGAGCGAGAAAGGCTTTAAAGTCA
TTTTTGGAATGTCTCTGGCGTTGTCTCCGCTTGTGTCAATTTTACAGAT
TATGTGGCCCATCTGACCCAACATAACCAGCAGCATCATTTGGTAACCAA
TCACATTTCGGTTGCTACATTTCTGACACCATCAGCGATGAGGTGGGCTA
CAACCATTGCTTGTTTGTTCGCCATTCTCTTGGCGATATGAGATGTTCTC
ACAAATCGGGGTGTTTCTTGACTCCGCACTCTTGCTTTTGGTGGTTTTT
TTGCTGTGTACCGGCTTGTCTGGTCCCTTTGCCGATGGCAACGGCGACAG
CTCGACATACCAATATATATATAACTTGACGATATGCGAGCTGAATGGGA
CCGAATGGCTGTCTGACCATTTTAATTGGGCAGTCGAGACTTTTGTGCTC
TACCCAGTGGCGACTCATATCCTCTCACTGGGTTTCTCACGACAAGTCA
TTTCCTTGATGCGTTCGGTCTTGGAGCTGTGTCAATTACAGGGTTTTGTG
GCGGGCGGTACGTGCTCAGCAGCGTGTACGGCGCTTGTGCACTAGCAGCG
CTCGTATGTTTTGTTATCCGTGCTGCCAAAAATTGTATGGCTTGCCGCTA
TGCTCGTACCCGGTTTACCAACTTCATTGTGGATGACCGGGGGAGAATCC
ATCGGTGGAGGTCTCCAATAGTGGTGGAAAAATTAGGTAAAGCTGACGTC
GGCGGCGACCTTGTCAACCATCAAACATGTCATCCTCGAAGGAGTCAAAGC
TCAACCCTTGACGAGGACTTCGGCCGAGCAATGGGAGGCCTAGATAATTT
CTGCAACGATCCCACCGCCGCACAAAAGCTTGTGCTAGCCTTTAGCATCA
CGTATACACCCATCATGATATACGCCCTTAAGGTGTACGCGGCCGACTC
TTGGGGTTGTTGCACATCTTGATATTTCTGAACTGTTCTTTCACGTTTCGG
ATACATGACATATATGCATTTTGAATCCACCAACCGTGTGCGGCTTACCA
TGGGGGGCGTTGTGCGCCCTTCTGTGGGGCGTTTATAGTTTCATAGAGTCA
TGGAAGTTTATCACTTCCAGATGCAGATTGTGTTGCCTAGGCCGGCGATA

FIG. 11

10/10**complete genome sequence for SEQ ID NO:5**

>96V198c1p49 complete genome passage 49 (SEQ ID NO:5)

CATTCTGGCCCCTGCCCACCACGTAGAAAGTGCTGCAGGCCTCCATCCGA
TCCCAGCGTCTGGTAACCAAGCATACGCTGTGAGAAAGCCCGGACTAACA
TCAGTGAACGGCACTCTGGTACCAGGACTTCGAGGCCTCGTGCTGGGCGG
CAAACGAGCTGTTAAACGAGGAGTGGTTAACCTCGTCAAGTATGGCCGGT
AAAAACCAGAGCCAGAAGAAAAAGAAAAATCCAGCTCCAATGGGGAATGG
CCAGTCAGTCAATCAACTGTGCCAGCTGCTGGGCACAATGATAAAGTCCC
AGCGCCAGCGACCCAGGGGAGGACAGGCTAAAAAGAAAAAGCCTGAGAAG
CCACATTTCCCCCTGGCTGCTGAAGATGACGTCCGGCACCATCTCACCCA
GACCGAGCGCTCCCTTTGCTTGCAATCGATCCAGACGGCTTTTAATCAAG
GCGCAGGAAGTGGCTCGCTTTCATCCAGCGGGAAGATCGGTTTTTCAGGTT
GAGTTTATGCTACCGGTTGCTCATAACAGTGCGCCTGATTCGCGTGACTTC
TACATCCGCCGGTCAGGATGCAAATTAATTTGATAGTCAGGTGAATGGCC
GCGATTGGCGTGTGGCCTCTGAGTCACCTATTCAATTAGGGCGATCACAT
GGGGGTCAGACTTAATTAGGCAGGAACCATGTGACCGAAATT

FIG. 1J

ZP000148A_seq_ST25.txt
SEQUENCE LISTING

<110> Zoetis Services LLC
Calvert, Jay Gregory
Balasch, Monica
Fort, Maria
Pearce, Douglas S.
Keith, Marcia L.

<120> EFFECTIVE VACCINATION AGAINST EUROPEAN STRAINS OF PORCINE
REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VIRUS PRIOR TO
WEANING

<130> ZP000148A PCT

<150> 62/434,144
<151> 2016-12-14

<160> 8

<170> PatentIn version 3.5

<210> 1
<211> 15092
<212> DNA
<213> Artificial Sequence

<220>
<223> PRRS virus isolate 96V198 - passage 0

<400> 1
atgatgtgta gggtattccc cctacataca cgacatttct agtgtttg tgcccttgag 60
gcgtgggtac agccccgccc cacctcttgg cccctgttct agcccaacag gtatccttct 120
ctctcggggc gagcgcgccg cctgctgctc ccttgacgca ggaaggacct ccgagattatt 180
tccggagagc acctgcttta cgggatctcc accctttaac catgtctggg acgttctccc 240
ggtgcatgtg cccccggct gcccggttat tttggaacgc cggccaagtc ttttgacac 300
ggtgtctcag tgcgcggtct cttctctctc cggagcttca ggacactgac ctgctgcaa 360
ttggcttggt ttacaagcct aaagacaagc ttcactggaa agtccctatc ggcatccctc 420
aggtggagtg tactccgtcc gggtgctggt ggctctcagc catcttcccc ttggcgcgca 480
tgacctccgg caatcacaac tttctccaac gacttggtgaa ggttgctgat gttttgtacc 540
gtgacggctg cttggcacct cgacaccttc gcgaactcca agtttacgag cgcggctgca 600
actggtaccc gatcacgggg cccgtgcccg ggatggggtt gtacgcgaac tccatgcacg 660

ZP000148A_seq_ST25.txt

tgtccgacca accgtttcct ggtgccactc atgtgttaac gaactcacct ctgcctcagc	720
aggcttgtcg acagccgttt tgtccatttg aggaggttca ttccgacgta tacagggtgga	780
agaaattcgt gattttttgtg gattcctctt ctgacggccg atctcgcagc atgtggacac	840
caggatccga cgactcggct gccttagaag tattgccacc tgaactagaa tgtcgagtcg	900
aaatcctcgt tcggagtttt cctgcccacc accctgtcga catcaccaac tgggagctca	960
ctgagtcccc tgagcacggc ttttccttca gcacgtctca ttcttgtggt taccttgccc	1020
aaaaccctga cgtgtttgat ggtaagtgtt ggctttcttg ctttttgggc ctgccgaccg	1080
aagtgtggcg tcatgaggag tatctagcta acgccttcgg ttaccaaac aagtggggcg	1140
tgcattgtaa gtacctccag cgcaggcttc aggtccgcgg catgcgtgct gtagttgatc	1200
ctgatggctc catccacgtt gaagcgtgtt cttgccccca gtcttggatc aggcacctga	1260
ctctaaatga tgacgtcacc ccaggatttg ttgcctaac atcccttcgc attgagccga	1320
acacagagcc tactactttc cggatctttc ggtttggagc gcataagtgg tatggcgtg	1380
ccggcaaacg ggctcgtgct aagcgtgccg ctaaagggtga gaagaattca gctcccaccc	1440
ccaaggctgc cctgccggct cccacctgtg gaattaccac ctactctcca ccgacagacg	1500
ggtcttgtgg ttggcatgtc cttgccgcca taatgaaccg gatgataaat ggtgacttca	1560
cgtcccctct gactccgtac aacagaccag aggatgattg ggcttctgat tatgatcttg	1620
ctcaggcaat ccaatgtcta caactgcctg ctaccgtggc ccggagtcgc gcctgtccta	1680
acgccaagta cctcataaaa ctcaacggag tccactggga ggtagagggt aggtcaggaa	1740
tggctcctcg ctctctttct cgtgaatgtg tggttggcgt ttgctctgaa ggttgtgttg	1800
caccgcctta tccagcagac gggctaccag aacgagcact cgaggccttg gcgtctgctt	1860
acaggttacc ctctgattgt gttagctctg gcattgctga ctttcttgct aaccacctc	1920
ctcaggaatt ctggaccctc gacaaaatgt tgacctcccc gtcaccagag cgggtccggc	1980
tctctagttt gtataaatta ctattagagg ttgttccgca aaaatgcggg gccacggaag	2040
gggctttcgt ctatgctgtt gagagaatgt tgaaggattg tccgagctcc aaacaggcca	2100
tggcccttct ggcaaaaatt aaagtccat cctcaaaggc cccgtctgtg tcccttgacg	2160
agtgtttccc tacggatgtt tcagccgact tcgagccagc atctcaggaa agggcccaaa	2220

ZP000148A_seq_ST25.txt

gttccggtgc tgctgttgtc ctgtgttcac cggaagtaaa agagttcgaa gaagcagccc	2280
cagaagaagt tcaagagagt ggccacaagg tcgcccgtc tgcatattgtt gccgagggtc	2340
ctaacaatga acaggtaccg atggctgccg gcgagcaact gaagcccggg ggtcgcgttt	2400
tggcggtcgg gaatgctcat gaagggtgtt tggcctcaac tagtccgact aacctgatag	2460
gcgggaactt ccccccttca gactccatga aagagagcat gctcaatagc tgggaagacg	2520
aaccactgga tttgtcccaa ccggcaccag ctgtttacaat gacccttgtg agagagcaaa	2580
cacccgacaa cctgggtcct gatgccggtg ctttccccgt caccgttcga aaatttgtcc	2640
cgacagggcc tacactccgt catgtttgagc actgcggcac ggagtcaggc gacagcagtt	2700
cgcccttgga tctgtcttat gcgcaaacc tggaccagcc tttaaatcta tccttggccg	2760
cttggccagt gagggccacc gcgtctgacc ctggctgggt ccacggtagg cgcgagccta	2820
tctttgtaaa gcctcgagat gctttctctg acggcgattc agcccttcag ttcggggagc	2880
tgtctgaatc cggctctgtc atcgagtttg accggacaag aaatgctccg gcggtcgacg	2940
cctctgttga cttgacggct tcgaacgagg ccctctctgt ggttgatcct ttcgaatttg	3000
ccgaactcaa gcgcccgcgt ttttccgcac aagccttaat tgaccgaggc ggtccacttg	3060
ccgacgtcca tgcaaaaata aagaaccggg tatatgaaca gtgcctccag gcttgtgagc	3120
ctggcagtcg cgcaaccca gccaccaagg agtggctcga taaaatgtgg gataggggtg	3180
acatgaagac ttggcgctgt acctcgcagt tccaagctgg tcgcattctt gcgtccctta	3240
aattcctccc tgacatgatt caggacacac cacctcctgt tcctaggaag aatcgggcta	3300
gtgataatgc cggcctgaag cgactgggtg cgcagtggga cagaaaattg agtgcaaccc	3360
ccccttcaaa accggttgga ccaacacttg accaaattgt cccttcgccc acgggtaccc	3420
agcaagaaga tgtcacttcc tccgatgggc catctcatgc gccggatcct cctagtcgaa	3480
tgagcacgag tgggagttgg aagggccttg tgctctctgg taccgtctc gcggggtcca	3540
ttagtcagca tttcatgaca tgggtttttg aggttttctc ccatctcca gcttttgcac	3600
tcacactttt ctcgccgagg ggctctatgg ctccaggtga ttggatgttt gcaggtgttg	3660
ttttacttgc tctcctgctc tgtcgttctt acccagtact cgggtgcctt cccttattgg	3720
gtgtcctttc tggctctgtg cggcgcgttc gtctgggggt ttttggttct tggatggctt	3780

ZP000148A_seq_ST25.txt

tcgctgtatt tttattctcg actccatcca acccagtcgg ttcttcttgt gaccacgatt	3840
cgccggagtg tcatgctgag cttttggctc ttgagcagcg ccaactttgg gaacctgtgc	3900
gcggccttgt ggtcggcccc tcgggcctct tatgtgtcat tcttggcaag ctactcggtg	3960
ggtcacgtta tctctggcat gttttcttac gtttatgcat gcttgcagat ttggcccttt	4020
ctcttgttta tgtggtgtcc caggggcgtt gtcacaagtg ttggggaaaag tgtataagaa	4080
cagctccggc ggaggtggct ctcaatgtgt tccctttctc gcgcgctacc cgtagctctc	4140
ttgtgtcctt gtgcgatcgg ttccaagcgc caaaaggggt tgatcctgtg tacttggcaa	4200
cgggttggcg cgggtgttgg tgtggtgaga gtccattca tcaaccgcac caaaaacca	4260
tagcttatgc taatttggat gaaaagaaaa tatctgcca aacggtgggt gctgtccac	4320
acgatcccag tcaggccatc aagtgcctga aagttttgca ggccgggagga gccatcgtgg	4380
accagcccac acctgaggtc gtccgtgtat ccgaaatccc cttctcagcc ccatttttcc	4440
caaaagttcc agtcaacca gattgtaagg ttgtggtgga ttcggacact tttgtggctg	4500
cggttcgctg cggctactcg acagcacaac tggtcctagg ccagggaac tttgccaagt	4560
taaatcagat tcccctcagg agctccatct ccaccaaagc gattggcggg gcctcttaca	4620
cccttgctgt ggctcaagtt tctgtgtgga ctcttgttca cttcatcctc ggtctttggt	4680
tcacgtcacc ccaagtgtgt ggccgaggaa cctctgacct atggtgttca aatccttttt	4740
cataccctac ctatggcccc ggggttgtgt gtcctctcg actttgtgtg tctgccgacg	4800
gggtcactct accattgttc tcagccgtgg ctcaactctc cggtagagag gtggggattt	4860
ttatttttgt gctcgtctcc ttgatggctt tggcccaccg catggccctt aaggcagaca	4920
tgttgatggg ctttctggct ttttgtgctt acgcctggcc catgagctcc tggttgattt	4980
gcttctttcc tacactcttg aagtgggtta ccctccaccc tcttactatg ctttgggtgc	5040
actcattctt ggtgttttgt ctgccagcag ccggcatcct ctactaggg ataactggcc	5100
ttctttgggc ggttggccgc tttactcagg ttgccggaat tattacacct tatgacatcc	5160
accagtacac ctctgggcca cgtggcgcag cagccgtggc cacagcccca gaaggcactt	5220
atatggccgc cgtcaggaga gctgctttaa ctgggcgaac tttaatcttc accccgtctg	5280
cagttggatc ctttctcgaa ggagctttca ggactcataa accttgtctt aacaccgtaa	5340

ZP000148A_seq_ST25.txt

atgttgtagg ctcttcctt ggttccggcg gggttttcac tattgacggc agaaaaattg	5400
ttgtcactgc tgcccatgtg ttgaacggcg acacagctag agtcaccggc gactcctaca	5460
accgcatgca tactttcaag accaatggtg attatgcctg gtcccatgct gatgactggc	5520
ggggcgttgc ccctgcggtc aaggttgcga aggggtaccg cggtcgtgcc tactggcaaa	5580
catcaactgg cgtcgaaccc ggtgttattg gggaagggtt cgccttctgt ttcaccacct	5640
gtggcgattc ggggtcaccg gtcattctag aatccggtga tctcattgga atccataccg	5700
gttcaaataa acttggttct ggtcttgtga caaccctga aggggagaca tgtaccatca	5760
aagaaaccaa gctctctgac ctttcagac atttcgcagg cccaagcgtt cctcttgggg	5820
atattaaatt gaggccagcc atcatccctg atgtaacatc cattccgagt gacttggcat	5880
cgctcctatc ctccgtccct gtagtggaag gcggcctctc gaccgttcaa cttttgtgtg	5940
tctttttcct actttggcgc atgatgggccc atgcctggac tcccattgtt gccgtgggtt	6000
tctttttact gaatgaaatt cttccagcag ttttgggtccg agccgtgttt tcttttgcac	6060
tttttgtgct tgcatgggccc accccctggt ctgcacagggt gttgatgatt agactcctca	6120
cggcatctct caaccgcaac aagctttctc tggcgttcta cgcactcggg ggggtcgtcg	6180
gtttggccgc tgaaatcggg acttttgcctg gcaaattgcc tgaattgtct caaacctttt	6240
cgacatactg cttcttacct agggtccttg ctatgaccag ttgtgttccc accatcatca	6300
ttggtggact ccatgccctc ggtgtaattc tgtggttggtt caaataaccgg tgcctccaca	6360
acatgctcgt tggatgagg agtttttcaa gcgccttctt cctacgggat tttgcagagg	6420
gtaatctcag aaaagggtgtt tcgcagtcct gtggcatgaa taacgagtcc ctgacggctg	6480
ctttagcttg caagttgtca caggctgacc ttgatttttt gtccagctta acgaacttca	6540
agtgccttgt atctgcttcg aatatgaaaa atgctgccgg ccagtacatt gaagcggcgt	6600
atgccaaggc cctgcgcaa gagttggcat ctctagttca gattgacaaa atgaaaggag	6660
ttttgtccaa actcgaggcc tttgctgaaa cagctactcc gtcccttgac ataggtgacg	6720
tgattgttct gcttgggcaa catccacacg gatctatcct tgatattaat gtggggactg	6780
agaggaaaac tgtgtccgtg caagagaccc ggagcctagg cggttccaaa ttcagtgttt	6840
gtactgtcgt gtctaacaca cccgtggacg ccttaaccgg catcccactc cagacaccaa	6900

ZP000148A_seq_ST25.txt

cccctctttt tgagaatggt ccgcgtcatc gcagcgaaga agacgatctt aaagtcgaga	6960
ggatgaagaa acactgtgtg tccctcggct tccacaacat caatggcaaa gtttactgca	7020
agatttgga caagtccacc ggtgacacct ttacacgga tgattcccgg tataccacg	7080
accatgcttt tcaggacagg tcagccgact acagagacag ggactatgag ggtgtgcaaa	7140
ccgccccca acagggattt gatccaaagt ctgaaacccc tgtcggcact gttgtgatcg	7200
gcggtattac gtataacagg tatctgacta agggtaagga gggtctgggt cccaagcccg	7260
acaattgcct tgaagctgcc aagctgtctc ttgagcaagc tctcgctggg atgggccaaa	7320
cttgcgacct tacagctgcc gaggtggaaa agttaaagcg catcatcagt caactccaag	7380
gtttgaccac tgaacaggct ttaaactgct agccgctagc ggcttgaccc gctgtggccg	7440
cggcggctta gttgttactg aaacggcggg aaaaattgta agataccaca gcagaacttt	7500
caccttgggc cttttggacc taaaagtcgc ttctgagggt gaggtgaaga gatcaactga	7560
gcagggccac gctgtttgtg caaacctatg ttctgggtgt gtattgatga gacctaccc	7620
accgtccctt gttgacgttc ttctgaaacc cggacttgac acaacacccg gcattcaacc	7680
ggggcatggg gccgggaata tgggcgtgga cggttccatt tgggattttg aaaccgcacc	7740
cacaaaggca gaactcgagt tgtccaagca aataatccaa gcatgtgaag tcaggcgcgg	7800
ggatgccccg aacctccaac tcccttaca gctctgtcca gttagggggg atcctgagcg	7860
gcataaaggc cgccttatca ataccagggt tggagatttg cttacaaga ctctcaaga	7920
caccaagtcc gcaatccacg cggcttggtt cctgcacccc aacggggctc ccgtgtctga	7980
tggtaaatcc acattaggca ccactcttca acacggtttc gagctttatg tccctactgt	8040
gccctatagt gtcattggagt accttgattc acgccctgac acccccttta tgtgtaccaa	8100
acatggtact cccgaggctg ctgcagagga ccttcgaaaa tatgacttat ccaccaagg	8160
gtttgtcctg cctggggctc tacgtctagt acgcagattc atctttggcc atattggtaa	8220
ggcaccgcca ctgttctctc catcaactta tccgccaag aactctatgg cagggatcaa	8280
tggccagagg ttcccaacaa aagacgttca gagcatacct gaaattgatg aaatgtgtgc	8340
ccgcgtgtc aaagagaatt ggcaaaactgt gacgccttgc accctcaaaa aacagtattg	8400
ttccaagcct aaaaccagga ccctccttgg taccaacaac ttcatcgctt tggctcacag	8460

ZP000148A_seq_ST25.txt

gtcggcactc agcgggtgtca ctcaggcggtt catgaaaaaa gcctggaagt ccccgatcgc	8520
cttggggaaa aacaaattta aggagttgca ctgcactgtc gccggtaggt gcctcgaggc	8580
cgacttggcc tcttgtgatc gcagcacccc ggccatcgtg aggtggtttg ttgccaacct	8640
cctgtacgaa cttgcgggtt gtgaagagta cttgccaagc tatgtgctca attgttgcca	8700
tgacctggtg gcaacgcaaa atggcgcctt caaaaaacgt ggtggcctgt catctggaga	8760
ccccgttacc agtgtgtcca acacagtgtt ttcactgggtg atttatgctc agcacatggt	8820
gctgtcggct ttgaagatgg gtcattgaaat cggcctcaag ttcctcgagg aacagcttaa	8880
gttcgaggac cttctcgaaa tccagcctat gttggtatat tctgatgacc ttgtattgta	8940
tgctgaaaga cccaccttcc ccaattacca ttggtgggtt gagcaccttg acctgatgct	9000
gggttttaag acagacccaa aaaagactgt cataactgac aaaccagct ttcttggtg	9060
tagaatcgaa gcggggcgac aactggttcc caatcgcgac cgcattctgg ctgctcttgc	9120
atatcacatg aaggcgcaga acgcctcaga gtattatgcg tctgctgccg cgattctgat	9180
ggactcgtgt gcttgcatth accacgatcc tgagtgggtat gaggacctca tctgcggcat	9240
tgctcgatgc gcccgccagg atggctacag tttcccaggc ccgccgttct tcatgtccat	9300
gtgggagaag ctgaaaagtc acaatgaagg caagaaattc cgccactgtg gcatttgtga	9360
cgccaaggcc gatcatgcgt ccgcctgttg gcttgattta tgtctgttcc actcgcactt	9420
tcatcaacac tgccctgtta ctctgagctg cggccatcat gccggttcaa aagaatgccc	9480
acagtgtcag tcacctgttg gagctggtaa atccccctt gatgcagtgc tgaaacaaat	9540
cccgtacaaa cccctcgtc ctgtcatcat gaagggtggac aataaaacaa cgacccttga	9600
cccgggaagg tatcagtccc gtcgaggtct tgttgctgtc aagaggggta ttgcaggcaa	9660
tgaggttgat ctcgctgatg gagactatca ggtagtgccc cttttgcca cctgcaaaga	9720
cataaacatg gtgaaggtgg ctgtcaatgt gctactcagc aagttcatag tggggccgcc	9780
aggttccggc aagacgacct ggctactgag tcaagttcag gatgatgatg tcatctatac	9840
acctacccat cagaccatgt tcgacatagt caatgccctc aaagtctgta ggtattccat	9900
cccagtggct tcagggtccc ctttcccacc gcccgccaga tctggaccgt gggttaggct	9960
cgttgctagc gggcacatcc ctggccgaat atcatacctt gacgaggccg gatattgcaa	10020

ZP000148A_seq_ST25.txt

tcatctggat attctcagac tgcttttctaa gacacctctt gtgtgttttg gtgaccttca	10080
gcaacttcac cctgtcggct ttgactctta ctgttatgtt tttgatcaaa tgcctcataa	10140
gcagctgacc actatttata gatttggccc caatatctgt tctgccattc aaccttggtta	10200
cagggaaaaa ctcgaatcta aggccaggaa cactaggggtg gtttttactg cccggcccgt	10260
ggcttttgggt caggtgttga cgccatatca taaagaccgc accggctcag ctataaccat	10320
agattcatcc caggagacca cttttagtgt tgtgacgctg catttgccgt cgccagattc	10380
cctgaacaaa tcccgggctc ttgtagctat tactcgggca aggcattgggt tgttcatcta	10440
tgacctcat aaccaactcc ggaagttttt caacttaaca cctgagcgca ctgattgcaa	10500
cctcgtgttc aaccgcgggg acgagttgggt agttttgaat gcagataatg cagtcacgac	10560
tgtggctaag gttttggagg cgggcccgtc tcggtttcga gtatcagatc caaggtgcaa	10620
gtctctttta gccgcttgct cggccagtct ggaaggaggc tgcatgccac tgccgcaagt	10680
ggcacataat ctggggtttt acttctctcc agatagtcca gcatttgcac ctctgcaaaa	10740
agagctggca ccacattggc cgggtggttac tagtcagaac aaccaggcat ggcccgaccg	10800
acttgtcgct agtatgcggc cagttgatgc ccgctacagc aagcctatgg tcggtgcagg	10860
gtatgtgggt gggccatcca ctttccttgg cactcctgggt gtagtgtcat actatctcac	10920
gctgtacatc aggggtgagc cccaggcctt gccggagaca ctcgtctcaa cgggacgtat	10980
agctactgat tgtcgagagt atctcgacgc agctgaggaa gaagcagcta aagaactccc	11040
tcacgcattc attggcgatg tcaaaggtac tacagtgggg ggggtgtcacc acattacgtc	11100
aaaatacctt cccaggctct tgcccaagga ctccgttgcc gtggtcggag tgagttcgcc	11160
cggcaaggct gccaaagccg tgtgtactct caccgatgtg tatcttcccg agtccggcc	11220
atatttgcaa ccggaaacag catcgaaatg ctggaaactt aaactagact tcagggatgt	11280
cagactgatg gtctggaaag gagccaccgc atatttccaa ctggaggggc tcacatggtc	11340
agcgctgccc gactatgcca ggttcattca gctacaaaa gatgccgttg tgtacattga	11400
tccgtgcata ggaccggcaa cagccaaccg taaggttgtg agaactacag attggcgagc	11460
tgacctggca gtgacaccgt acgactacgg tgctcagagc attttgacta cagcctgggt	11520
cgaggacctt gggccgcagt ggaagatttt agggttgcaa cccttcaagc gggcatttgg	11580

ZP000148A_seq_ST25.txt

ctttgaaaac actgaggatt gggcgatcct tgcacgtcgc atgaacgacg gcaaggacta	11640
cactgactat aactggaatt gtgttcgaca acgcccacat gccatctacg gacgtgccccg	11700
tgaccatacg tatcactttg cccctggcac tgaactgcaa gtagagctag gtaaaccctg	11760
gctaccgcct gagcaagtac cgtgaatcta gagtgatgca atgggggtcac tgtggagtaa	11820
aatcagtcaa ctgttcgtgg atgccttcac tgagttcctc gttagtgtgg ttgatattgt	11880
catcttcctt gccatactgt ttgggttcac cgttgcaggg tggttactgg tctttttttt	11940
cagagtgggtt tgctccgcgc ttctccgttc gcgctctgcc attcactctt ccgaactatc	12000
gaaggtccta tgagggttg ctacctaatt gcagaccgga tgttccacaa ttcgcagtca	12060
agcacccatt ggggtatactt tggcacatgc gagtttccca cttaatgac gaaatgggtct	12120
ctcgccgcat ttaccagacc atggaacatt caggtcaagc ggcttggaag caggtgggta	12180
gtgaagctac tctcacaaga ctgtcaaagc tcgacatagt tctccacttc caacacctgg	12240
ccgcaataga ggcggattct tgccgcttcc tcagctcacg acttgatgct ctgaaaaatc	12300
ttgccgttgg caatatgagc ctacagtaca acaccacgtt ggaccgcgtt gagctcatct	12360
tcccaacacc aggtacgagg cccaaattga ccgatttttag acaatggctc gtcagtgtgc	12420
acgcttctat tttttcctct gtggcctcat cagttacctt gttcatagtg ctttggcttc	12480
gaattccagc tctacgctat gtttttgggt tccattggcc cacggcaaca catcattcga	12540
gctaaccatc aattacacta tatgtaagcc ctgtctcacc agtcaagcgg ctcgacaaaag	12600
gctcgaacc ggtcgcaaca tgttgtgcaa aataggacac accacgtgtg aggagcgtga	12660
ccatgatgag ttgtcaatga ccattccgtc cgggtacgat aacctcaaac ttgagggtta	12720
ttacgcttgg ctggcttttt tgtccttttc ttacgcagcc caatttcac cagagttggt	12780
cggaataggg aatgtgtcac gcgtcttcgt ggataaacgg caccagttca tctgtgccga	12840
gcacgacgga caaaattcaa ccgtatccac cgaacataat atttccgcat tgtatgcggc	12900
gtactaccac caccaggtag acgggggcaa ttggttccac ctggaatggc tgcggccttt	12960
cttttcctcc tggctagtag tcaatatattc atggtttctg aggcgttcgc ctgcaagccc	13020
tgtttctcga cgcatttatc agatattgag accaacacga ccgcggctgc cggtttcatg	13080
gtccttcagg acatcaactg tttccacagt ggctcagagg cacaaacgac tgggtcccatc	13140

ZP000148A_seq_ST25.txt

agaaagtcgt	cccaatgccg	tgaagccgtc	ggcactcccc	agtacatcaa	gataacggcc	13200
aatgtgaccg	acgaatcata	cttgtacaac	gcggacttgc	tgatgctttc	tgctgcctt	13260
ttctacgcct	cagagatgag	cgagaaaggc	tttaaagtca	tttttgggaa	tgtctctggc	13320
gttgtctccg	cttgtgtcaa	ttttacagat	tatgtggccc	atgtgaccca	acatacccag	13380
cagcatcatt	tggtaatcaa	tcacattcgg	ttgctacatt	tcctgacacc	atcagcaatg	13440
aggtagggcta	caaccattgc	ttgtttgttc	gccattctct	tggcgatatg	agatgttctc	13500
acaaatcggg	gtgtttcttg	actccgcact	cttgcttctg	gtggcttttt	ttgctgtgta	13560
ccggccttgc	ctggctcttt	gccgatggca	acggcaacag	ctcgacatac	caatatatat	13620
ataacttgac	gatatgcgag	ctgaatggga	ccgaatggct	gtccgaccat	tttaattggg	13680
cagtcgagac	ttttgtgctc	taccagtggt	cgactcatat	cctctcactg	ggtttcctca	13740
cgacaagtca	tttccttgat	gcgttcggtc	ttggagctgt	gtcagttaca	gggttttgtg	13800
gcgggaggta	cgtgctcagc	agcgtgtacg	gcgcttgtgc	actagcagcg	ctcgtatgtt	13860
ttgttatccg	tgctgcaaaa	aattgtatgg	cttgccgcta	tgctcgtacc	cggtttacca	13920
acttcattgt	ggatgaccgg	gggagaatcc	atcggtaggag	gtctccaata	gtggtggaaa	13980
aattaggtaa	agctgacgtc	ggcggcgacc	ttgtcaccat	caaacatgtc	atcctcgaag	14040
gagtcaaagc	tcaacccttg	acgaggactt	cggccgagca	atgggaggcc	tagataattt	14100
ctgcaacgat	cccaccgccg	cacaaaagct	tgtgctagcc	tttagcatca	catatacacc	14160
catcatgata	tacgccctta	aggtgtcacg	cggccgactc	ttggggttgt	tgacatctt	14220
gatatttctg	aactgttctt	tcacgttcgg	atacatgaca	tatatgcatt	ttgaatccac	14280
caaccgtgtc	gcgcttacca	tggggggcgt	tgtcgccctt	ctgtggggcg	tttatagttt	14340
catagagtca	tggaagttta	tcacttccag	atgcagattg	tgttgcctag	gccggcgata	14400
cattctggcc	cctgcccacc	acgtagaaag	tgctgcaggc	ctccatccga	tcccagcgtc	14460
tggttaacaa	gcatacgctg	tgagaaagcc	cggactaaca	tcagtgaacg	gcactctggg	14520
accaggactt	cgaggcctcg	tgctgggcgg	caaacgagct	gttaaacgag	gagtgggttaa	14580
cctcgtcaag	tatggccggt	aaaaaccaga	gccagaagaa	aaagaaaaat	ccagctccaa	14640
tggggaatgg	ccagtcagtc	aatcaactgt	gccagctgct	gggcacaatg	ataaagtccc	14700

ZP000148A_seq_ST25.txt

agcgccagcg acccagggga ggacaggcta aaaagaaaaa gcctgagaag ccacatttcc	14760
ccctggctgc tgaagatgac gtccggcacc atctcaccca gaccgagcgc tccctttgct	14820
tgcaatcgat ccagacggct tttaatcaag gcgcaggaac tgcgtcgctt tcatccagcg	14880
ggaagatcgg ttttcagggtt gagtttatgc taccggttgc tcatacagtg cgcctgattc	14940
gcgtgacttc tacatccgcc ggtcaggatg caaattaatt tgatagtcag gtgaatggcc	15000
gcgattggcg tgtggcctct gagtcaccta ttcaattagg gcgatcacat gggggtcaga	15060
cttaattagg caggaacat gtgaccgaaa tt	15092

<210> 2

<211> 15092

<212> DNA

<213> Artificial Sequence

<220>

<223> PRRS virus isolate 96V198 - passage 1

<400> 2

atgatgtgta gggatttccc cctacatata cgacacttct agtgtttgtg tgccttggag	60
gcgtgggtac agccccgccc cacctcttgg cccctgttct agcccaacag gtatccttct	120
ctctcggggc gagcgcgccg cctgctgctc ccttgacgca ggaaggacct cccgagtatt	180
tccggagagc acctgcttta cgggatctcc accctttaac catgtctggg acgttctccc	240
ggtgcatgtg caccctggct gcccggttat tttggaacgc cggccaagtc ttttgacac	300
ggtgtctcag tgcgcggtct cttctctctc cagagcttca ggacactgac ctcgctgcaa	360
tcggcttggt ttacaagcct aaagacaagc ttcactggaa agtccctatc ggcatccctc	420
aggtggagtg tactccgtcc ggggtctgtt ggctctcagc catcttcgtc ttggcgcgca	480
tgacctccgg caatcacaac tttccccaac gacttggtgaa ggttgctgat gttttgtacc	540
gtgacggctg cttggcacct cgacaccttc gcgaactcca agtttacgag cgcggctgca	600
actggtaccc gatcacgggg cccgtgcccg ggatgggttt atacgcgaac tctatgcatg	660
tgtccgacca accgtttcct ggtgccaccc atgtgttgac gaactcacct ctgcctcagc	720
aggcttgtcg acagccgttt tgtccatttg aggaggttca ttccgacgta tacaggtgga	780
agaaattcgt gatTTTTTgtg gattcctctt ctgacggccg atctcgcag atgtggacac	840

ZP000148A_seq_ST25.txt

caggatccga cgactcggct gccttagaag tattgccacc tgaactagaa tgtcgagtcg	900
aaatcctcgt tcggagtttt cctgcccacc accctgtcga catcaccaac tgggagctca	960
ctgagtcccc tgagcacggt ttttccttca gcacgtctca ttcttgtggt taccttgccc	1020
aaaaccctga cgtgtttgat ggtaagtgtt ggctttcttg ctttttgggc ctgccgaccg	1080
aagtgtggcg tcatgaggag tatctagcta acgccttcgg ttaccaaacc aagtggggcg	1140
tgcatggtaa gtacctccag cgcaggcttc aggtccgcgg catgcgtgct gtagttgatc	1200
ctgatgggcc catccacgtt gaagcgtgtt cttgccccca gtcttggatc aggcacctga	1260
ctctaaatga tgacgtcacc ccaggatttg ttgcctaac atcccttcgc attgagccga	1320
acacagagcc tactactttc cggatctttc ggtttggagc gcataagtgg tatggcgctg	1380
ccggcaaacc ggctcgtgct aagcgtgccg ctaaagggtga gaagaattca gctcccacc	1440
ccaaggtcgc cctgccgggc cccacctgtg gaattaccac ctactctcca ccgacagacg	1500
ggtcttgtgg ttggcatgtc cttgccgcca taatgaaccg gatgataaat ggtgacttca	1560
cgtcccctct gactccgtac aacagaccag aggatgattg ggcttctgat tatgatcttg	1620
ctcaggcaat ccaatgtcta caactgcctg ctaccgtggt ccggagtcgc gcctgtccta	1680
acgccaagta cctcataaaa ctcaacggag tccactggga ggtagagggt aggtcaggaa	1740
tggctcctcg ctctctttct cgtgaatgtg tggttggcgt ttgctctgaa ggttgtgttg	1800
caccgcctta tccagcagac gggctaccag aacgagcact cgaggccttg gcgtctgctt	1860
acaggttacc ctctgattgt gttagctctg gcattgctga ctttcttgct aaccacctc	1920
ctcaggaatt ctggaccctc gacaaaatgt tgacctcccc gtcaccagag cggtcgggt	1980
tctctagttt gtataaatta ctattagagg ttgttccgca aaaatgcggt gccacggaag	2040
gggctttcgt ctatgctgtt gagagaatgt tgaaggattg tccgagctcc aaacaggcca	2100
tggcccttct ggcaaaaatt aaagtccat cctcaaaggc cccgtctgtg tcccttgacg	2160
agtgtttccc tacggatgtt tcagccgact tcgagccagc atctcaggaa aggccccaaa	2220
gttccgggtgc tgctgttgct ctgtgttcac cggaagtaaa agagtctgaa gaagcagccc	2280
cagaagaagt tcaagagagt ggccacaagg tcgcccgtc tgcatattgtt gccgagggtc	2340
ctaacaatga acaggtaccg atggctgccg gcgagcaact gaagcccgggt ggtcgcgttt	2400

ZP000148A_seq_ST25.txt

tggcggtcgg gaatgctcat gaaggtgttc tggcctcaac tagttcgact aacctgatag	2460
gcgggaactt ccccccttca gactccatga aagagagcat gctcaatagc tgggaagacg	2520
aaccactgga tttgtcccaa ccggcaccag ctgttacaat gacccttggtg agagagcaaa	2580
cacccgacaa cctgggtcct gatgccggtg ctttccccgt caccgttcga aaatttgtcc	2640
cgacagggcc tacactccgt catgttgagc actgcggcac ggagtcaggc gacagcagtt	2700
cgcccttgga tctgtcttat gcgcaaacc tggaccagcc tttaaatcta tccttggccg	2760
cttggccagt gagggccacc gcgtctgacc ctggctgggt ccacggtagg cgtgagccta	2820
tctttgtaaa gcctcgagat gctttctctg acggcgattc agcccttcag ctcggggagc	2880
tgtctgaatc cggctccgtc atcgagtttg accggacaag aaatgctccg gcggtcgacg	2940
cccctgttga cttgacggct tcgaacgagg ccctctctgt ggttgatcct ttcgaatttg	3000
ccgaactcaa gcgcccgcgt ttttccgcac aagccttaat tgaccgaggc ggtccacttg	3060
ccgacgtcca tgcaaaaata aagaaccggg tatatgaaca gtgcctccag gcttgtgagc	3120
ctggcagtcg cgcaacccca gccaccaagg agtggctcga taaaatgtgg gatagggtgg	3180
acatgaagac ttggcgctgt acctcgcagt tccaagctgg tcgcattctt gcgtccctta	3240
aattcctccc tgacatgatt caggacacac cacctcctgt tcctaggaag aatcgggcta	3300
gtgataatgc cggcctgaag cgactgggtg cgcagtgga cagaaaattg agtgcaaccc	3360
ccccttcaaa accggttgga ccaacacttg accaaattgt cccttcgccc acgggtaccc	3420
agcaagaaga tgtcacttcc tccgatgggc catctcatgc gccggatcct cctagtcgaa	3480
tgagcacgag tgggagttgg aagggccttg tgctctctgg taccgtctc gcggggtcca	3540
ttagtcagca tttcatgaca tgggtttttg aggttttctc ccatctccca gcttttgcac	3600
tcacactttt ctcgccgagg ggctctatgg ctccaggtga ttggatgttt gcaggtgttg	3660
ttttacttgc tctcctgctc tgtcgttctt acccagtact cgggtgcctt cccttattgg	3720
gtgtcctttc tggctctgtg cggcgcgttc gtctgggggt ttttggttct tggatggctt	3780
tcgctgtatt tttattctcg actccatcca acccagtcgg ttcttcttgt gaccacgatt	3840
cgccggagtg tcatgctgag cttttggctc ttgagcagcg ccaactttgg gaacctgtgc	3900
gcggccttgt ggtcggcccc tcgggcctct tatgtgtcat tcttggcaag ctactcggtg	3960

ZP000148A_seq_ST25.txt

ggtcacgtta tctctggcat gttttcttac gtttatgcat gcttgacagat ttggcccttt	4020
ctcttgttta tgttggtgtcc caggggctgt gtcacaagtgt ttggggaaaag tgtataagaa	4080
cagctccggc ggaggtggct ctcaatgtgt tccctttctc gcgcgctacc cgtagctctc	4140
ttgtgtcctt gtgcgatcgg ttccaagcgc caaaaggggt tgatcctgtg tacttggcaa	4200
cgggttggcg cgggtgttgg tgttggtgaga gtcccattca tcaaccgcac caaaaacca	4260
tagcttatgc taatttggat gaaaagaaaa tatctgcccc aacgggtgggt gctgtccac	4320
acgatcccag tcaggccatc aagtgcctga aagttttgca ggccgggagga gccatcgtgg	4380
accagcccac acctgaggtc gtccgtgtat ccgaaatccc cttctcagcc ccatttttcc	4440
caaaagttcc agtcaacca gattgtaagg ttgtggtgga ttcggacact tttgtggctg	4500
cggttcgtg cggctactcg acagcacaac tggtcctagg ccagggaac tttgccaagt	4560
taaatcagat tcccctcagg agctccatct ccaccaaagc gattggcggg gcctcttaca	4620
cccttgctgt ggctcaagtt tctgtgtgga ctcttgttca cttcatcctc ggtctttggt	4680
tcacgtcacc ccaagtgtgt ggccgaggaa cctctgacct atggtgttca aatccttttt	4740
cataccctac ctatggcccc ggggttgtgt gtcctctctg actttgtgtg tctgccgacg	4800
gggtcactct accattgttc tcagccgtgg ctcaactctc cggtagagag gtggggattt	4860
ttattttggt gtcgtctcc ttgatggctt tggcccaccg catggccctt aaggcagaca	4920
tgttgatggg ctttctggct ttttgtgctt acgcctggcc catgagctcc tggttgattt	4980
gcttctttcc tacactcttg aagtgggtta ccctccacct tcttactatg ctttgggtgc	5040
actcattctt ggtgttttgt ctgccagcag ccggcatcct ctcaataggg ataactggcc	5100
ttctttgggc ggttggccgc tttactcagg ttgccggaat tattacacct tatgacatcc	5160
accagtacac ctctgggcca cgtggcgctg cagctgtggc cacagcccca gaaggcactt	5220
atatggccgc cgtccggaga gctgctttta ctgggcgaac tttaatcttc acccgtctg	5280
cagttggatc ctttctcgaa ggagctttca ggactcataa acctgtctt aacaccgtaa	5340
atgttgtagg ctcttcctt ggttccggcg gggttttcac tattgacggc agaaaaattg	5400
ttgtcactgc tgcccatgtg ttgaacggcg acacagctag agtcaccggc gactcctaca	5460
accgcatgca tactttcaag accaatggtg attatgcctg gtcccatgct gatgactggc	5520

ZP000148A_seq_ST25.txt

ggggcgttgc ccctgcggtc aaggttgcga aggggtaccg cggtcgtgcc tactggcaaa	5580
catcaactgg cgtcgaaccc ggtgttattg gggaagggtt cgcttctgt ttcaccacct	5640
gtggcgattc ggggtcaccg gtcattctcag aatccggtga tctcattgga atccataaccg	5700
gttcaaataa acttggttct ggtcttgtga caaccctga aggagagaca tgtgccatca	5760
aagaaaccaa gctctctgac ctttccagac atttcgcagg cccaagcgtt cctcttgggg	5820
atattaaatt gagtccagcc atcatccctg atgtaacatc cattccgagt gacttggcat	5880
cgctcctatc ctccgtccct gtagtggaag gcggcctctc gaccgttcaa cttttgtgtg	5940
tctttttcct actttggcgc atgatgggccc atgcctggac tcccattgtt gccgtggggt	6000
tctttttact gaatgaaatt cttccagcag ttttgggtccg agccgtgttt tcttttgcac	6060
tttttgtgct tgcatgggccc acccctgggt ctgcacaggt gttgatgatt agactcctca	6120
cggcatctct caaccgcaac aagctttctc tggcgttcta cgcactcggg ggggtcgtcg	6180
gtttggccgc tgaaatcggg acttttgcgt gcaaattgcc tgaattgtct caaaccttt	6240
cgacatactg cttcttacct agggtccttg ctatgaccag ttgtgttccc accatcatca	6300
ttggtggact ccatgccctc ggtgtaattc tgtgtgtgtt caaataccgg tgcctccaca	6360
acatgctcgt tggatgatggg agtttttcaa gcgccttctt cctacgggat tttgcagagg	6420
gtaatctcag aaaagggtgtt tcgcagtcct gtggcatgaa taacgagtcc ctgacggctg	6480
ctttagcttg caagttgtca caggctgacc ttgatttttt gtccagctta acgaacttca	6540
agtgtttgt atctgcttcg aatatgaaaa atgctgccgg ccagtacatt gaagcggcgt	6600
atgccaaggc cctgcgcaa gagttggcat ctctagttca gattgacaaa atgaaaggag	6660
ttttgtccaa actcgaggcc tttgctgaaa cagctactcc gtcccttgac ataggtgacg	6720
tgattgttct gcttgggcaa catccacacg gatctatcct tgatattaat gtgggggactg	6780
agaggaaaac tgtgtccgtg caagagaccc ggagcctagg cggttccaaa ttcagtgttt	6840
gtactgtcgt gtctaacaca cccgtggacg ccttaaccgg catcccactc cagacaccaa	6900
cccctctttt tgagaatggt ccgcgtcatc gcagcgaaga agacgatctt aaagtcgaga	6960
ggatgaagaa acactgtgtg tccctcggct tccacaacat caatggcaaa gtttactgca	7020
agatttggga caagtccacc ggtgacacct ttacacgga tgattcccgg tatacccacg	7080

ZP000148A_seq_ST25.txt

accatgcttt tcaggacagg tcagccgact acagagacag ggactatgag ggtgtgcaaa	7140
ccgcccccca acagggatatt gatccaaagt ctgaaacccc tgtcggcact gttgtgatcg	7200
gcggtattac gtataacagg tatctgacta agggtaagga ggttctgggt cccaagcccc	7260
acaattgcct tgaagctgcc aagctgtctc ttgagcaagc tctcgctggg atgggcaaaa	7320
cttgcgacct tacagctgcc gaggtggaaa agttaaacg catcatcagt caactccaag	7380
gtttgaccac tgaacaggct ttaaactgct agccgctagc ggcttgaccc gctgtggccg	7440
cggcggtta gttgttactg aaacggcggg aaaaattgta agataccaca gcagaacttt	7500
caccttgggc ctttggacc taaaagtcgc ttctgagggt gaggtgaaga gatcaactga	7560
gcagggccac gctgttgtgg caaacctatg ttctgggtgt gtattgatga gacctaccc	7620
accgtccctt gttgacgttc ttctgaaacc cggacttgac acaacacccg gcattcaacc	7680
ggggcatggg gccgggaata tgggcgtgga cggttccatt tgggatattt aaaccgcacc	7740
cacaaaggca gaactcgagt tgtccaagca aataatccaa gcatgtgaag tcaggcgcgg	7800
ggatgccccg aacctccaac tcccttaca gctctgtcca gttagggggg atcctgagcg	7860
gcataaaggc cgccttatca ataccagggt tggagatttg ccttacaaga ctctcaaga	7920
caccaagtcc gcaatccacg cggcttggtt cctgcacccc aacggggctc ccgtgtctga	7980
tggtaaattc acattaggca ccactcttca acacgggttc gagctttatg tccctactgt	8040
gccctatagt gtcattgagt accttgattc acgccctgac acccccttta tgtgtacaa	8100
acatggtact cccgaggctg ctgcagagga ccttcgaaaa tatgacttat ccaccaagg	8160
gtttgtcctg cctggggctc tacgtctagt acgcagattc atctttggcc atattggtaa	8220
ggcaccgcca ctgttcctc catcaactta tcccgccaag aactctatgg cagggatcaa	8280
tggccagagg ttcccaacaa aagacgttca gagcatacct gaaattgatg aaatgtgtgc	8340
ccgcgctgtc aaagagaatt ggcaaaactgt gacgccttgc accctcaaaa aacagtattg	8400
ttccaagcct aaaaccagga ccattccttg taccaacaac ttcattgcct tggctcacag	8460
gtcggcactc agcgggtgtca ctcaggcggt catgaaaaaa gcctggaagt ccccgatcgc	8520
cttggggaaa aacaaattta aggagttgca ctgcactgtc gccggtaggt gcctcgaggc	8580
cgacttggcc tcttgtgatc gcagcacccc ggccatcgtg aggtgggttg ttgccaacct	8640

ZP000148A_seq_ST25.txt

cctgtacgaa cttgcgggtt gtgaagagta cttgccaagc tatgtgctca attgttgcca	8700
tgacctgggtg gcaacgcaaa atggcgcctt caaaaaacgt ggtggcctgt catctggaga	8760
ccccgttacc agtgtgtcca acacagtgtg ttcactgggtg atttatgctc agcacatggg	8820
gctgtcggct ttgaagatgg gtcattgaaat cggcctcaag ttcctcgagg aacagcttaa	8880
gttcgaggac cttctcgaaa tccagcctat gttggtatat tctgatgacc ttgtattgta	8940
tgctgaaaga cccaccttcc ccaattacca ttggtgggtt gagcaccttg acctgatgct	9000
gggttttaag acagacccaa aaaagactgt cataactgac aaaccagct ttcttggtg	9060
tagaatcgaa gcggggcgac aactggttcc caatcgcgac cgcattctgg ctgctcttgc	9120
atatcacatg aaggcgcaga acgcctcaga gtattatgctg tctgctgccg cgattctgat	9180
ggactcgtgt gcttgcatg accacgatcc tgagtgggtat gaggacctca tctgcggcat	9240
tgctcgatgc gcccgccagg atggctacag tttcccaggc ccgccgttct tcatgtccat	9300
gtgggagaag ctgaaaagtc acaatgaagg caagaaattc cgccactgtg gcatttgtga	9360
cgccaaggcc gatcatgcgt ccgcctgtgg gcttgattta tgtctgttcc actcgcactt	9420
tcatcaacac tgccctgtta ctctgagctg cggccatcat gccggttcaa aagaatgcc	9480
acagtgtcag tcacctgttg gagctggtaa atccccctt gatgcagtgc tgaaacaaat	9540
cccgtacaaa cccctcgtc ctgtcatcat gaaggtggac aataaaacaa cgacccttga	9600
cccgggaagg tatcagtccc gtcgaggtct tgttgctgtc aagaggggta ttgcaggcaa	9660
tgaggttgat ctgctgatg gagactatca ggtagtgccc cttttgcaa cctgcaaaga	9720
cataaacatg gtgaaggtgg ctgtcaatgt gctactcagc aagttcatag tggggccgcc	9780
aggttccggc aagacgacct ggctactgag tcaagttcag gatgatgatg tcatctatac	9840
acctacccat cagaccatgt tcgacatagt caatgccctc aaagtctgta ggtattccat	9900
cccagtggct tcagggtccc ctttcccacc gcccgccaga tctggaccgt gggtaggct	9960
cgttgctagc gggcacatcc ctggccgaat atcatacctt gacgaggccg gatattgcaa	10020
tcatctggat attctcagac tgctttctaa gacacctctt gtgtgtttgg gtgaccttca	10080
gcaacttcac cctgtcggct ttgactctta ctgttatgtt tttgatcaaa tgcctcataa	10140
gcagctgacc actatttata gatttggccc caatatctgt tctgccattc aaccttgta	10200

ZP000148A_seq_ST25.txt

cagggaaaaa ctcgaatcta aggccaggaa cactagggtg gtttttactg cccggcccgt	10260
ggcttttgggt caggtgttga cgccatatca taaagaccgc accggctcag ctataaccat	10320
agattcatcc cagggagcca cctttgatgt tgtgacgctg catttgccgt cgccagattc	10380
cctgaacaaa tcccgggctc ttgtagctat tactcgggca aggcatgggt tgttcatcta	10440
tgaccctcat aaccaactcc ggaagttttt caacttaaca cctgagcgca ctgattgcaa	10500
cctcgtgttc aaccgcgggg acgagttgggt agttttgaat gcagataatg cagtcacgac	10560
tgtggctaag gttttggagg cgggcccgtc tcggtttcga gtatcagatc caaggtgcaa	10620
gtctctttta gccgcttgct cggccagtct ggaaggaggc tgcatgccac tgccgcaagt	10680
ggcacataat ctgggggtttt acttctctcc agatagtcca gcatttgcac ctctgcaaaa	10740
agagctggca ccacattggc cgggtggttac tagtcagaac aaccaggcat ggcccgaccg	10800
acttgtcgct agtatgcggc cagttgatgc ccgctacagc aagcctatgg tcggtgcagg	10860
gtatgtgggtt gggccatcca ctttccttgg cactcctgggt gtagtgtcat actatctcac	10920
gctgtacatc aggggtgagc cccaggcctt gccggagaca ctcgtctcaa cgggacgtat	10980
agctactgat tgtcgagagt atctcgacgc agctgaggaa gaagcagcta aagaactccc	11040
tcacgcattc attggcgatg tcaaaggtac tacagtgggg ggggtgtcacc acattacgtc	11100
aaaatacctt cccaggctct tgcccaagga ctccgttgcc gtggtcggag tgagttcgcc	11160
cggcaaggct gccaaagccg tgtgtactct caccgatgtg tatcttcccg agtccggcc	11220
atatttgcaa ccggaacag catcgaaatg ctggaaactt aaactagact tcagggatgt	11280
cagactgatg gtctggaaag gagccaccgc atattttcaa ctggaggggc tcacatggtc	11340
agcgctgccc gactatgcca ggttcattca gctacaaaa gatgccgttg tgtacattga	11400
tccgtgcata ggaccggcaa cagccaaccg taaggttgtg agaactacag attggcgagc	11460
tgacctggca gtgacaccgt acgactacgg tgctcagagc attttgacta cagcctgggt	11520
cgaggacctt gggccgcagt ggaagatttt agggttgcaa cccttcaagc gggcatttgg	11580
ctttgaaaac actgaggatt gggcgatcct tgcacgtcgc atgaacgacg gcaaggacta	11640
cactgactat aactggaatt gtgttcgaca acgcccacat gccatctacg gacgtgcccg	11700
tgaccatacg tatcactttg cccctggcac tgaactgcaa gtagagctag gtaaaccgcc	11760

ZP000148A_seq_ST25.txt

gctaccgcct gagcaagtac cgtgaatcta gagtgatgca atggggtcac tgtggagtaa	11820
aatcagtcaa ctgttcgtgg atgccttcac tgagttcctc gttagtgtgg ttgatattgt	11880
catcttcctt gccatactgt ttgggttcac cgttgcaggg tggttactgg tctttttttt	11940
cagagtgggt tgctccgcgc ttctccgttc gcgctctgcc attcactctt ccgaactatc	12000
gaaggctcta tgagggcttg ctacctaatt gcagaccgga tgttccacaa ttcgcagtca	12060
agcaccatt ggggtatactt tggcacatgc gagtttccca cttaattgac gaaatgggtct	12120
ctcgccgcat ttaccagacc atggaacatt caggtcaagc ggcctggaag caggtgggta	12180
gtgaagctac tctcacaaga ctgtcaaagc tcgacatagt tctccacttc caacacctgg	12240
ccgcaataga ggcggattct tgccgcttcc tcagctcacg acttgtgatg ctgaaaaatc	12300
ttgccgttgg caatatgagc ctacagtaca acaccacgtt ggaccgcgtt gagctcatct	12360
tcccaacacc aggtacgagg cccaaattga ccgattttag acaatggctc gtcagtgtgc	12420
acgcttctat tttttcctct gtggcctcat cagttacctt gttcatagtg ctttggcttc	12480
gaattccagc tctacgctat gtttttggtt tccattggcc cacggcaaca catcattcga	12540
gctaaccatc aattacacta tatgtaagcc ctgtctcacc agtcaagcgg ctcgacaaag	12600
gctcgaaccc ggtcgcaaca tgtggtgcaa aataggacac accacgtgtg aggagcgtga	12660
ccatgatgag ttgtcaatga ccattccgtc cgggtacgat aacctcaaac ttgagggtta	12720
ttacgcttgg ctggcttttt tgtccttttc ttacgcagcc caatttcac cagagtgtgt	12780
cggaataggg aatgtgtcac gcgtcttcgt ggataaacgg caccagttca tctgtgccga	12840
gcacgacgga caaaattcaa ccgtatccac cgaacataat atttccgcat tgtatgcggc	12900
gtactaccac caccaggtag acgggggcaa ttggttccac ctggaatggc tgcggccttt	12960
cttttcctcc tggctagtac tcaatatattc atggtttctg aggcgttcgc ctgcaagccc	13020
tgtttctcga cgcatttatc agatattgag accaacacga ccgcggctgc cggtttcatg	13080
gtccttcagg acatcaactg tttccacagt ggctcagagg cacaacgac tgggtccatc	13140
agaaagtcgt cccaatgccg tgaagccgtc ggactcccc agtacatcaa gataacggcc	13200
aatgtgaccg acgaatcata cttgtacaac gcggacttgc tgatgctttc tgcgtgcctt	13260
ttctacgcct cagagatgag cgagaaaggc tttaaagtca tttttgggaa tgtctctggc	13320

ZP000148A_seq_ST25.txt

gttgtctccg ctttgtgtcaa ttttacagat tatgtggccc atgtgaccca acatacccag	13380
cagcatcatt tggtaatcaa tcacattcgg ttgctacatt tcctgacacc atcagcaatg	13440
aggtgggcta caaccattgc ttgtttgttc gccatttctt tggcgatatg agatgtttctc	13500
acaaatcggg gtgtttcttg actccgcact cttgcttctg gtggcttttt ttgctgtgta	13560
ccggcttgtc ctggctcttt gccgatggca acggcaacag ctcgacatac caatatatat	13620
ataacttgac gatatgcgag ctgaatggga ccgaatggct gtccgaccat ttaaatggg	13680
cagtcgagac ttttgtgctc taccagtggt cgactcatat cctctcactg ggtttcctca	13740
cgacaagtca tttccttgat gcgttcggtc ttggagctgt gtcagttaca gggttttgtg	13800
gcgggcggtg cgtgctcagc agcgtgtacg gcgcttgtgc actagcagcg ctcgtatggt	13860
ttgttatccg tgctgcaaaa aattgtatgg cttgccgcta tgctcgtacc cggtttacca	13920
acttcattgt ggatgaccgg gggagaatcc atcggtgagg gtctccaata gtggtggaaa	13980
aattaggtaa agctgacgtc ggcggcgacc ttgtcaccat caaacatgtc atcctcgaag	14040
gagtcaaagc tcaacccttg acgaggactt cggccgagca atgggaggcc tagataattt	14100
ctgcaacgat cccaccgccg cacaaaagct tgtgctagcc tttagcatca catatacacc	14160
catcatgata tacgccctta aggtgtcacg cggccgactc ttgggggttg tgcatatctt	14220
gatatttctg aactgttctt tcacgttcgg atacatgaca tatatgcatt ttgaatccac	14280
caaccgtgtc gcgcttacca tggggggcgt tgctgccctt ctgtggggcg tttatagttt	14340
catagagtca tggaagttta tcacttccag atgcagattg tgttgccctag gccggcgata	14400
cattctggcc cctgcccacc acgtagaaag tgctgcaggc ctccatccga tcccagcgtc	14460
tggtaaccaa gcatacgctg tgagaaagcc cggactaaca tcagtgaacg gcactctggt	14520
accaggactt cgaggcctcg tgctgggcgg caaacgagct gttaaacgag gagtgggttaa	14580
cctcgtcaag tatggccggt aaaaaccaga gccagaagaa aaagaaaaat ccagctccaa	14640
tggggaatgg ccagtcagtc aatcaactgt gccagctgct gggcacaatg ataaagtccc	14700
agcgccagcg acccagggga ggacaggcta aaaagaaaaa gcctgagaag ccacatttcc	14760
ccctggctgc tgaagatgac gtccggcacc atctcaccca gaccgagcgc tccctttgct	14820
tgcaatcgat ccagacggct tttaatcaag gcgcaggaac tgcgtcgctt tcatccagcg	14880

ZP000148A_seq_ST25.txt

ggaagatcgg ttttcagggtt gagtttatgc taccggttgc tcatacagtg cgcctgattc	14940
gcgtgacttc tacatccgcc ggtcaggatg caaattaatt tgatagtcag gtgaatggcc	15000
gcgattggcg tgtggcctct gagtcaccta ttcaattagg gcgatcacat gggggtcaga	15060
cttaattagg caggaacccat gtgaccgaaa tt	15092

<210> 3
 <211> 15092
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> PRRS virus isolate 96V198 - passage 9

<400> 3	
atgatgtgta gggatttccc cctacatata cgacacttct agtgtttgtg tgccttggag	60
gcgtgggtat agccccgccc cacctcttgg cccctgttct agcccaacag gtatccttct	120
ctctcggggc gagcgcgccg cctgctgctc ccttgcagca ggaaggacct cccgagtatt	180
tccggagagc acctgcttta cgggatctcc accctttaac catgtctggg acgttctccc	240
ggatgcatgtg cccccggct gcccggtat tttggagcgc cggccaagtc tattgcacac	300
gggtgtctcag tgcacggtct cttctctctc cggagcttca ggacactgac ctcgctgcaa	360
ttggcttgtt ttacaagcct aaagacaagc ttacttgga agtccctatc ggcatccctc	420
aggtggagtg tactccgtcc ggggtgctgtt ggctctcagc catcttcccc ttggcgcgca	480
tgacctccgg caatcacaac tttctccaac gacttgtgaa ggttgctgat gttttgtacc	540
gtgacggctg cttggcacct cgacaccttc gcgaactcca agtttacgag cgcggctgca	600
actggtaccc gatcacgggg cccgtgcccg ggatgggttt gtacgcgaac tccatgcacg	660
tgtccgacca accgtttcct ggtgccgctc atgtgttaac gaactcacct ctgcctcagc	720
aggcttgtcg acagccgttt tgtccatttg aggaggttca ttccgacgta tacagggtga	780
agaaattcgt gattttttgtg gattcctctt ctgacggccg atctcgcag atgtggacac	840
caggatccga cgactcggct gccttagaag tattgccacc tgaactagaa tgtcgagtcg	900
aaatcctcgt tcggagtttt cctgcccacc accctgtcga catcaccaac tgggagctca	960
ctgagtcccc tgagcacggt ttttcttca gcacgtctca ttcttgtggt taccttgccc	1020

ZP000148A_seq_ST25.txt

aagaccctga cgtgtttgat ggtaagtgtt ggctttcttg ctttttgggc ctgccgaccg	1080
aagtgtggcg tcatgaggag tatctagcta acgccttcgg ttaccaaacc aagtggggcg	1140
tgcatggtaa gtacctccag cgcaggcttc aggtccgcgg catgcgtgct gtagttgatc	1200
ctgatgggcc catccacgtt gaagcgtgtt cttgccccca gtcttgatc aggcacctga	1260
ctctaaatga tgacgtcacc ccaggatttg ttgcctaacc atcccttcgc attgagccga	1320
acacagagcc tactactttc cggatctttc gggttgagc gcataagtgg tatggcgtg	1380
ccggcaaacc ggctcgtgct aagcgtgccg ctaaagggtga gaagaattca gctcccacc	1440
ccaaggctgc cctgccggtc cccacctatg gaattaccac ctactctcca ccgacagacg	1500
ggctctgttg ttggcatgtc cttgccgcca taatgaaccg gatgataaat ggtgacttca	1560
cgtcccctct gactccgtac aacagaccag aggatgattg ggcttctgat tatgatcttg	1620
ctcaggcaat ccaatgtcta caactgcctg ctaccgtggc ccggagtcgc gcctgtccta	1680
acgccaagta cctcataaaa ctcaacggag tccactggga ggtagagggt aggtcaggaa	1740
tggctcctcg ctctctttct cgtgaatgtg tggttggcgt ttgctctgaa ggttgtgttg	1800
caccgcctta tccagcagac gggctaccag aacgagcact cgaggccttg gcgtctgctt	1860
acaggttacc ctctgattgt gttagctctg gcattgctga ctttcttgct aaccacctc	1920
ctcaggaatt ctggaccctc gacaaaatgt tgacctccc gtcaccagag cggctccggct	1980
tctctagttt gtataaatta ctattagagg ttgttccgca aaaatgcggg gccacggaag	2040
gggctttcgt ctatgctgtt gagagaatgt tgaaggattg tccgagctcc aaacaggcca	2100
tggcccttct ggcaaaaatt aaagtccat cctcaaaggc cccgtctgtg tcccttgacg	2160
agtgtttccc tacggatgtt tcagccgact tcgagccagc atctcaggaa aggcccaaa	2220
gttccgggtg tgctgttgtc ctgtgttcac cggaagtaaa agagtctgaa gaagcagccc	2280
cagaagaagt tcaagagagt ggccacaagg tcgcccgtc tgcatttggt gccgagggtc	2340
ctaacaatga acaggtaccg atggctgccg gcgagcaact gaagcccggg ggtcgcgttt	2400
tggcggtcgg gaatgctcat gaagggtgtt tggcctcaac tagttcgact aacctgatag	2460
gcgggaactt cccccctca gactccatga aagagagcat gctcaatagc tgggaagacg	2520
aaccactgga tttgtccaa ccggcaccag ctgttacaat gacccttggt agagagcaaa	2580

ZP000148A_seq_ST25.txt

cacccgacaa cctgggtcct gatgccggtg ctttccccgt caccgttcga aaatttgtcc	2640
cgacagggcc tacactccgt catgttgagc actgcggcac ggagtcaggc gacagcagtt	2700
cgcccttgga tctgtcttat gcgcaaacc tggaccagcc tttaaatcta tccttggccg	2760
cttggccagt gagggccacc gcgtctgacc ctggctgggt ccacggtagg cgtgagccta	2820
tctttgtaaa gcctcgagat gctttctctg acggcgattc agcccttcag ctcggggagc	2880
tgtctgaatc cggctccgtc atcgagtttg accggacaag aaatgctccg gcggtcgacg	2940
cccctgttga cttgacggct tcgaacaagg ccctctctgt ggttgatcct ttcgaatttg	3000
ccgaactcaa gcgcccgcgt ttttccgcac aagccttaat tgaccgaggc ggtccacttg	3060
ccgacgtcca tgcaaaaata aagaaccggg tatatgaaca gtgcctccag gcttgtgagc	3120
ctggcagtcg cgcaaccca gccaccaaga agtggctcga taaaatgtgg gatagggtgg	3180
acatgaagac ttggcgctgt acctcgcagt tccaagctgg tcgcattctt gcgtccctta	3240
aattcctccc tgacatgatt caggacacac cacctcctgt tcctaggaag aatcgggcta	3300
gtgataatgc cggcctgaag cgactgggtg cgcagtggga cagaaaattg agtgcaaccc	3360
ccccttcaaa accggttgga ccaacacttg accaaattgt cccttcgccc acgggtaccc	3420
agcaagaaga tgtcacttcc tccgatgggc catctcatgc gccggatcct cctagtcgaa	3480
tgagcacgag tgggagttgg aagggccttg tgctctctgg taccctctc gcgggggtcca	3540
ttagtcagca tttcatgaca tgggtttttg aggttttctc ccatctcca gcttttgac	3600
tcacactttt ctcgccgagg ggctctatgg cttcaggtga ttggatgttt gcagggtgtg	3660
ttttacttgc tctcctgctc tgtcgttctt acccagtatt cgggtgcctt cccttattgg	3720
gtgtcctttc tggctctgtg cggcgcgttc gtctgggggt ttttggttct tggatggctt	3780
tcgctgtatt tttattctcg actccatcca acccagtcgg ttcttcttgt gaccacgatt	3840
cgccggagtg tcatgctgag cttttggctc ttgagcagcg ccaactttgg gaacctgtgc	3900
gcggccttgt ggtcggcccc tcgggcctct tatgtgtcat tcttggaag ctactcgggtg	3960
ggtcacgtta tctctggcat gttttcttac gtttatgcat gcttgcagat ttggcccttt	4020
ctcttgttta tgtggtgtcc caggggcgtt gtcacaagtg ttggggaaag tgtataagaa	4080
cagctccggc ggaggtggct ctcaatgtgt tccctttctc gcgcgctacc cgtagctctc	4140

ZP000148A_seq_ST25.txt

ttgtgtcctt gtgcgatcgg ttccaagcgc caaaaggggt tgatcctgtg tacttggcaa	4200
cgggttggcg cgggtgttgg tgtggtgaga gtcccattca tcaaccgcac caaaaaccca	4260
tagcttatgc taatctggat gaaaagaaaa tatctgcccc aacggtgggt gctgtccac	4320
acgatcccag tcaggccatc aagtgccctga aagttttgca ggccgggagga gccatcgtgg	4380
accagcccac acctgaggtc gtccgtgtat ccgaaatccc cttctcagcc ccatttttcc	4440
caaaagttcc agtcaaccca gattgtaagg ttgtggtgga ttcggacact tttgtggctg	4500
cggttcgtg cggctactcg acagcacaac tggctcttagg ccagggaac tttgccaagt	4560
taaatcagat tcccctcagg agctccatct ccaccaaagc gattggcggg gcctcttaca	4620
cccttgctgt ggctcaagtt tctgtgtgga ctcttgttca cttcatcctc ggtctttggt	4680
tcacgtcacc ccaagtgtgt ggccgaggaa cctctgactc atggtgttca aatccttttt	4740
cataccctac ctatggcccc ggggttgtgt gctcctctcg actttgtgtg tctgccgacg	4800
gggtcactct accattgttc tcagccgtgg ctcaactctc cggtagagag gtggggattt	4860
ttattttggt gctcgtctcc ttgatggctt tggcccaccg catggccctt aaggcagaca	4920
tgttgatggt ctttctggct ttttgtgctt acgcctggcc catgagctcc tggttgattt	4980
gcttctttcc tacactcttg aagtgggtta ccctccaccc tcttactatg ctttgggtgc	5040
actcattctt ggtgttttgt ctgccagcag ccggcatcct ctactaggg ataactggcc	5100
ttctttgggc ggttggccgc tttactcagg ttgccggaat tattacacct tatgacatcc	5160
accagtacac ctctgggcca cgtggcgctg cagctgtggc cacagcccca gaaggcactt	5220
atatggccgc cgtccggaga gctgctttta ctgggcgaac tttaatcttc accccgtctg	5280
cagttggatc ctttctcgaa ggagctttca ggactcataa accttgtctt aacaccgtaa	5340
atgttgtagg ctcttcctt ggttcggcg gggttttcac tattgacggc agaaaaattg	5400
ttgtcactgc tgcccatgtg ttgaacggcg acacagctag agtcaccggc gactcctaca	5460
accgcatgca tactttcaag accaatggtg attatgcctg gtcccatgct gatgactggc	5520
ggggcgttgc ccctgcggtc aaggttgcca aggggtaccg cggctgtgcc tactggcaaa	5580
catcaactgg cgtcgaaccc ggtgttattg gggaagggtt cgccttctgt ttcaccacct	5640
gtggcgattc ggggtcacc gtcattctcag aatccggtga tctcattgga atccataccg	5700

ZP000148A_seq_ST25.txt

gttcaaataa acttggttct ggtcttgtga caaccctga aggggagaca tgtaccatca	5760
aagaaaccaa gctctctgac ctctccagac atttcgcagg cccaagcgtt cctcttgggg	5820
atattaaatt gagtccagcc atcatccctg atataacatc cattccgagt gacttggcat	5880
cgctcctatc ctccgtccct gtagtggaag gcggcctctc gaccgttcaa cttttgtgtg	5940
tctttttcct actttggcgc atgatgggcc atgcctggac tcccattgtt gccgtgggtt	6000
tctttttact gaatgaaatt cttccagcag ttttgggtccg agccgtgttt tcttttgcac	6060
tttttgtgct tgcatgggcc accccctggt ctgcacaggt gttgatgatt agactcctca	6120
cggcatctct caaccgcaat aagctttctc tggcgttcta cgcactcggg ggggtcgtcg	6180
gtttggccgc tgaaatcggg acttttgcctg gcaaattgcc tgaattgtct caaaccttt	6240
cgacatactg cttcttacct agggtccttg ctatgaccag ttgtgttccc accatcatca	6300
ttggtggact ccatgccctc ggtgtaattc tgtggttggt caaataccgg tgcctccaca	6360
acatgctcgt tggatgatggg agtttttcaa gcgccttctt cctacggat tttgcagagg	6420
gtaatctcag aaaaggtgtt tcgcagtcct gtggcatgaa taacgagtcc ctgacggctg	6480
ctttggcttg caagttgtca cgggctgacc ttgatTTTTT gtccagctta acgaacttca	6540
agtgccttgt atctgcttcg aatatgaaaa atgctgccgg ccagtacatt gaagcggcgt	6600
atgccaaggc cctgcgcaa gagttggcgt ctctagtcca gattgacaaa atgaaaggag	6660
ttttgtccaa actcgaggcc tttgctgaaa cagctactcc gtcccttgac ataggtgacg	6720
tgattgttct gcttgggcaa catccacacg gatctatcct tgatattaat gtggggactg	6780
agaggaaaac tgtgtccgtg caagagaccc ggagcctagg cggttccaaa ttcagtgttt	6840
gtactgtcgt gtctaacaca cccgtggacg ccttaaccgg catcccactc cagacaccaa	6900
cccctctttt tgagaatggt ccgcgtcatc gcagcgaaga agacgatctt aaagtcgaga	6960
ggatgaagaa aactgtgtg tccctcggct tccacaacat caatggcaaa gtttactgca	7020
agatttggga caagtccacc ggtgacacct ttacacgga tgattcccgg tataccacg	7080
accatgctct tcaggacagg tcagccgact acagagacag ggactatgag ggtgtgcaaa	7140
ccgccccca acagggattt gatccaaagt ctgaaacccc tgtcggcact gttgtgatcg	7200
gcggtattac gtataacagg tatctgacta agggtaagga gggtctgggt cccaagccc	7260

ZP000148A_seq_ST25.txt

acaattgcct tgaagctgcc aagctgtctc ttgagcaagc tctcgctggg atgggccaaa	7320
cttgcgacct tacagctgcc gaggtggaaa agttaagcg catcatcagt caactccaag	7380
gtttgaccac tgaacaggct ttaaactgct agccgctagc ggcttgaccc gctgtggccg	7440
cggcggctta gttgttactg aaacggcggg aaaaattgta agataccaca gcagaacttt	7500
caccttgggc ctttggacc taaaagtcgc ttctgaggtg gaggtgaaga gatcaactga	7560
gcagggccac gctgttgtgg caaacctatg ttctgggtgtt gtattgatga gacctcacc	7620
accgtccctt gttgacgttc ttctgaaacc cggacttgac acaacacccg gcattcaacc	7680
ggggcatggg gccgggaata tgggcgtgga cggttccatt tgggattttg aaaccgcacc	7740
cacaaaggca gaactcgagt tgtccaagca aataatccaa gcatgtgaag tcaggcgcgg	7800
ggatgccccg aacctccaac tcccttaca gctctgtcca gttagggggg atcctgagcg	7860
gcataaaggc cgccttatca ataccaggtt tggagatttg cttacaaga ctcctcaaga	7920
caccaagtcc gcaatccacg cggcttgttg cctgcacccc aacggggctc ccgtgtctga	7980
tggtaaatcc acattaggca ccactcttca acacggtttc gagctttatg tccctactgt	8040
gccctatagt gtcatggagt accttgattc acgccctgac acccccttta tgtgtaccaa	8100
acatggtact cccgaggctg ctgcagagga cttcgaaaa tatgacttat ccaccaagg	8160
gtttgtcctg cctgggggtc tacgtctagt acgcagattc atctttggcc atattggtaa	8220
ggcaccgcca ctgttcctc catcaactta tcccgccaag aactctatgg cagggatcaa	8280
tggccagagg ttcccaacaa aagacgttca gagcatacct gaaattgatg aaatgtgtgc	8340
ccgcgctgtc aaagagaatt ggcaaaactgt gacgccttgc accctcaaaa aacagtattg	8400
ttccaagcct aaaaccagga ccatccttgg taccaacaac ttcatgtcct tggctcacag	8460
gtcggcactc agcgggtgtca ctcaggcggt catgaaaaaa gcctggaagt ccccgatcgc	8520
cttggggaaa aacaaattta aggagttgca ctgcactgtc gccggtaggt gcctcgaggc	8580
cgacttggcc tcttgtgatc gcagcacccc ggccatcgtg aggtggtttg ttgccaacct	8640
cctgtacgaa cttgcgggtt gtgaagagta cttgccaagc tatgtgctca attgttgcca	8700
tgacctggtg gcaacgcaaa atggcgcctt caaaaacgt ggtggcctgt catctggaga	8760
ccccgttacc agtgtgtcca acacagtgt ttcactgggt atttatgctc agcacatggt	8820

ZP000148A_seq_ST25.txt

gctgtcggct ttgaagatgg gtcatgaaat cggcctcaag ttcctcgagg aacagcttaa	8880
gttcgaggac cttctcgaaa tccagcctat gttggtatat tctgatgacc ttgtattgta	8940
tgctgaaaga cccaccttcc ccaattacca ttggtgggtt gagcaccttg acctgatgct	9000
gggttttaag acagacccaa aaaagactgt cataactgac aaaccagct ttcttggctg	9060
tagaatcgaa gcggggcgac aactggttcc cagtcgcgac cgcatcttgg ctgctcttgc	9120
atatcacatg aaggcgcaga acgcctcaga gtattatgcg tctgctgccg cgattctgat	9180
ggactcgtgt gcttgcatg accacgatcc tgagtgggtat gaggacctca tctgcggcat	9240
tgctcgatgc gcccgccagg atggctacag tttcccaggc ccgccgttct tcatgtccat	9300
gtgggagaag ctgaaaagtc acaatgaagg caagaaattc cgccactgtg gcattttgtga	9360
cgccaaggcc gatcatgcgt ccgcctgtgg gcttgattta tgtctgttcc actcgcactt	9420
tcatcaacac tgccctgtta ctctgagctg cggccatcat gccggttcaa aagaatgccc	9480
acagtgtcag tcacctgttg gagctggtaa atccccctt gatgcagtgc tgaaacaaat	9540
cccgtaaaa cccctcgtc ctgtcatcat gaaggtggac aataaaacaa cgacccttga	9600
cccggaagg tatcagtccc gtcgaggtct tgttgctgtc aagaggggta ttgcaggcaa	9660
tgaggttgat ctgctgatg gagactatca ggtggtgccc cttttgcaa cctgcaaaga	9720
cataaacatg gtgaagggtg ctgtcaatgt gctactcagc aagttcatag tggggccgcc	9780
aggttccggc aagacgacct ggctactgag tcaagttcag gatgatgatg tcatctatac	9840
acctacccat cagaccatgt tcgacatagt caatgccctc aaagtctgta ggtattccat	9900
cccagtggct tcagggtccc ctttcccacc gcccgcaga tctggaccgt gggttaggct	9960
cgttgctagc gggcacatcc ctggccgaat atcatacctt gacgaggccg gatattgcaa	10020
tcatctggat attctcagac tgctttctaa gacacctctt gtgtgtttgg gtgaccttca	10080
gcaacttcac cctgtcggct ttgactctta ctgttatgtt tttgatcaaa tgcctcataa	10140
gcagctgacc actatttata gatttggccc caatatctgt tctgccattc aaccttgta	10200
cagggaaaaa ctcgaatcta aggccaggaa cactagggtg gtttttactg cccggcccgt	10260
ggcttttgggt caggtgttga cgccatatca taaagaccgc accggctcag ctataacat	10320
agattcatcc caggagcca cctttgatgt tgtgacgctg catttgccgt cgccagattc	10380

ZP000148A_seq_ST25.txt

cctgaacaaa tcccgggctc ttgtagctat cactcgggca aggcattgggt tgttcattcta	10440
tgaccctcat aaccaactcc ggaagttttt caacttaaca cctgagcgca ctgattgcaa	10500
cctcgtgttc aaccgcgggg acgagttgggt agttttgaat gcagataatg cagtcacgac	10560
tgtggctaag gttttggagg cgggcccgtc tcggtttcga gatatcagatc caagggtcaa	10620
gtctctttta gccgcttgct cggccagtct ggaaggaggc tgcatgccac tgccgcaagt	10680
ggcacataat ctgggggtttt acttctctcc agatagtcca gcatttgcac ctctgcaaaa	10740
agagctggca ccacattggc cggttgttac tagtcagaac aaccaggcat ggcccgaccg	10800
acttgtcgct agtatcggc cagttgatgc ccgctacagc aagcctatgg tcggtgcagg	10860
gtatgtgggtt gggccatcca ctttccttgg cactcctgggt gtagtgtcat actatctcac	10920
gctgtacatc aggggtgagc cccaggcctt gccggagaca ctcgtctcaa cgggacgtat	10980
agctactgat tgtcgagagt atctcgacgc agctgaggaa gaagcagcta aagaactccc	11040
tcacgcattc attggcgatg tcaaaggtac tacagtgggg ggggtgtcacc acattacgtc	11100
aaaatacctt cccaggctct tgcccaagga ctccgttgcc gtggtcggag tgagttcgcc	11160
cggcaaggct gccaaagccg tgtgtactct caccgatgtg tatcttcccg agtccggcc	11220
atatttgcaa ccggaacag catcgaaatg ctggaaactt aaactagact tcagggatgt	11280
cagactgatg gtctggaaag gagccaccgc atattttcaa ctggaggggc tcacatggtc	11340
agcgtgccc gactatgcca ggttcattca gctacaaaa gatgccgttg tgtacattga	11400
tccgtgcata ggaccggcaa cagccaaccg taaggttgtg agaactacag attggcgagc	11460
tgacctggca gtgacaccgt acgactacgg tgctcagagc attttgacta cagcctgggt	11520
cgaggacctt gggccgcagt ggaagatfff agggttgcaa cccttcaagc gggcatttgg	11580
ctttgaaaac actgaggatt gggcgatcct tgcacgtcgc atgaacgacg gcaaggacta	11640
cactgactat aactggaatt gtgttcgaca acgcccacat gccatctacg gacgtgcccg	11700
tgaccatacg tatcactttg cccctggcac tgaactgcaa gtagagctag gtaaaccg	11760
gctaccgcct gagcaagtac cgtgaatcta gtagtgatgca atggggtcac tgtggagtaa	11820
aatcagtcaa ctgttcgtgg atgccttcac tgagttcctt gttagtgtgg ttgatattgt	11880
catcttcctt gccatactgt ttgggttcac cgttgcaggg tggttactgg tctttttttt	11940

ZP000148A_seq_ST25.txt

cagagtgggtt	tgctccgcg	ttctccgttc	gcgctctgcc	attcactctt	ccgaactatc	12000
gaaggtccta	tgagggttg	ctacctaatt	gcagaccgga	tgttccacaa	ttcgcatcca	12060
agcaccatt	gggtatgttt	tggcacatgc	gagtttccca	cttaattgac	gaaatggctt	12120
ctcgccgcat	ttaccagacc	atggaacatt	caggtcaagc	ggcctggaag	caggtgggta	12180
gtgaagctac	tctcacaaga	ctgtcaaagc	tcgacatagt	tctccacttc	caacacctgg	12240
ccgcaataga	ggcggattct	tgccgcttcc	tcagctcacg	acttgtgatg	ctgaaaaatc	12300
ttgctgttgg	caatatgagc	ctacagtaca	acaccacgtt	ggaccgcgtt	gagctcatct	12360
tcccaacacc	aggtacgagg	cccaaattga	ccgatttttag	acaatggctc	gtcagtggtc	12420
acgcttctat	tttttcctct	gtggcctcat	cagttacctt	gttcataagt	ctttggcttc	12480
gaattccagc	tctacgctat	gtttttgggt	tccattggcc	cacggcaaca	catcattcga	12540
gctaaccatc	aattactcta	tatgtaagcc	ctgtctcacc	agtcaagcgg	ctcgacaaaag	12600
gctcgaacc	ggtcgcaaca	tgtgggtgca	aataggacac	accacgtgtg	aggagcgtga	12660
ccatgatgag	ttgtcaatga	ccattccgtc	cgggtacgat	aacctcaaac	ttgagggtta	12720
ttacgcttgg	ctggcttttt	tgtccttttc	ttacgcagcc	caatttcac	cagagttgtt	12780
cggaataggg	aatgtgtcac	gcgtcttcgt	ggataaacgg	caccagttca	tctgtgccga	12840
gcacgacgga	caaaattcaa	ccgtatccac	cgaacataat	atttccgcat	tgtatgcggc	12900
gtactaccac	caccaggtag	acgggggcaa	ttggttccac	ctggaatggc	tgcggccttt	12960
cttttcctcc	tggctagtac	tcaatatatt	atggtttctg	aggcgttcgc	ctgcaagccc	13020
tgtttctcga	cgcatttatc	agatatgtag	accaacacga	ccgcggctgc	cggtttcatg	13080
gtccttcagg	acatcaactg	tttccacagt	ggctcagagg	cacaaacgac	tgggtcccatc	13140
agaaagtcgt	cccaatgccg	tgaagccgtc	ggcactcccc	agtacatcaa	gataacggcc	13200
aatgtgaccg	acgaatcata	cttgtacaac	gcggacttgc	tgatgctttc	tgcgtgcctt	13260
ttctacgcct	cagagatgag	cgagaaaggc	tttaaagtca	tttttgggaa	tgtctctggc	13320
gttgtctccg	cttgtgtcaa	ttttacagat	tatgtggccc	atctgaccca	acatacccag	13380
cagcatcatt	tggtaaccaa	tcacattcgg	ttgctacatt	tcctgacacc	atcagcaatg	13440
aggtgggcta	caaccattgc	ttgtttgttc	gccattctct	tggcgatatg	agatgttctc	13500

ZP000148A_seq_ST25.txt

acaaatcggg	gtgtttcttg	actccgcact	cttgcttttg	gtggtttttt	ttgctgtgta	13560
ccggccttg	ctggctcctt	gccgatggca	acggcaacag	ctcgacatac	caatatatat	13620
ataacttgac	gatatgcgag	ctgaatggga	ccgaatggct	gtccgaccat	tttaattggg	13680
cagtcgagac	ttttgtgctc	taccagtggt	cgactcatat	cctctcactg	ggtttcctca	13740
cgacaagtca	tttccttgat	gcgttcggtc	ttggagctgt	gtcaattaca	gggttttgtg	13800
gcgggcggta	cgtgctcagc	agcgtgtacg	gcgcttggtc	actagcagcg	ctcgtatggt	13860
ttgttatccg	tgctgccaaa	aattgtatgg	cttgccgcta	tgctcgtacc	cggtttacca	13920
acttcattgt	ggatgaccgg	gggagaatcc	atcggtgagg	gtctccaata	gtggtggaaa	13980
aattaggtaa	agctgacgtc	ggcggcgacc	ttgtcaccat	caaacatgtc	atcctcgaag	14040
gagtcaaagc	tcaacccttg	acgaggactt	cggccgagca	atgggaggcc	tagataattt	14100
ctgcaacgat	cccaccgccg	cacaaaagct	tgtgctagcc	tttagcatca	cgtatacacc	14160
catcatgata	tacgccctta	aggtgtcacg	cggccgactc	ttggggttgt	tgcacatctt	14220
gatatttctg	aactgttctt	tcacgttcgg	atacatgaca	tatatgcatt	ttgaatccac	14280
caaccgtgtc	gcgcttacca	tggggggcgt	tgctgccctt	ctgtggggcg	tttatagttt	14340
catagagtca	tggaagttta	tcacttccag	atgcagattg	tgttgcctag	gccggcgata	14400
cattctggcc	cctgcccacc	acgtagaaaag	tgctgcaggc	ctccatccga	tcccagcgtc	14460
tggtaaccaa	gcatacgctg	tgagaaagcc	cggactaaca	tcagtgaacg	gcactctggt	14520
accaggactt	cgaggcctcg	tgctgggcgg	caaacgagct	gttaaacgag	gagtgggtta	14580
cctcgtcaag	tatggccggt	aaaaaccaga	gccagaagaa	aaagaaaaat	ccagctccaa	14640
tggggaatgg	ccagtcagtc	aatcaactgt	gccagctgct	gggcacaatg	ataaagtccc	14700
agcgccagcg	accaggggga	ggacaggcta	aaaagaaaaa	gcctgagaag	ccacatttcc	14760
ccctggctgc	tgaagatgac	gtccggcacc	atctcaccca	gaccgagcgc	tccctttgct	14820
tgcaatcgat	ccagacggct	tttaatcaag	gcgcaggaac	tgctgcgctt	tcatccagcg	14880
ggaagatcgg	ttttcagggt	gagtttatgc	taccggttgc	tcatacagtg	cgcttgattc	14940
gcgtgacttc	tacatccgcc	ggtcaggatg	caaattaatt	tgatagtcag	gtgaatggcc	15000
gcgattggcg	tgtggcctct	gagtcaccta	ttcaattagg	gcgatcacat	gggggtcaga	15060

cttaattagg caggaacccat gtgaccgaaa tt 15092

<210> 4

<211> 15092

<212> DNA

<213> Artificial Sequence

<220>

<223> PRRS virus isolate 96V198 - passage 14

<400> 4

atgatgtgta ggggtattccc cctacatata cgacacttct agtgtttgtg tgccttggag	60
gcggtgggtat agccccgccc cacctcttgg cccctgttct agcccaacag gtatccttct	120
ctctcggggc gagcgcgccg cctgctgctc ccttgacgca ggaaggacct cccgagtatt	180
tccggagagc acctgcttta cgggatctcc accctttaac catgtctggg acgttctccc	240
ggtgcatgtg caccggtt gcccgggtat tttggagcgc cggccaagtc tattgcacac	300
ggtgtctcag tgcacggtct cttctctctc cggagcttca ggacactgac ctgctgcaa	360
ttggcttggt ttacaagcct aaagacaagc ttcactggaa agtccctatc ggcatccctc	420
agggtggagt tactccgtcc ggggtgctgtt ggctctcagc catcttcccc ttggcgcgca	480
tgacctccgg caatcacaac tttctccaac gacttgtgaa ggttgctgat gttttgtacc	540
gtgacggctg cttggcacct cgacaccttc gcgaactcca agtttacgag cgcggctgca	600
actggtaccc gatcacgggg cccgtgcccg ggatggggtt gtacgcgaac tccatgcacg	660
tgtccgacca accgttccct ggtgccgctt atgtgttaac gaactcacct ctgcctcagc	720
aggcttgtcg acagccgttt tgtccatttg aggaggttca ttccgacgta tacagggtga	780
agaaattcgt gatTTTTgtg gattcctctt ctgacggccg atctcgcatg atgtggacac	840
caggatccga cgactcggct gccttagaag tattgccacc tgaactagaa tgtcgagtcg	900
aaatcctcgt tcggagtttt cctgcccacc acctgtgcga catcaccaac tgggagctca	960
ctgagtcctc tgagcacggt ttttccttca gcacgtctca ttcttggtgt taccttgccc	1020
aagaccctga cgtgtttgat ggtaagtgtt ggctttcttg ctttttgggc ctgccgaccg	1080
aagtgtggcg tcatgaggag catctagcta acgccttcgg ttaccaaacc aagtggggcg	1140
tgcattgtaa gtacctccag cgcaggcttc aggtccgcgg catgcgtgct gtagttgatc	1200

ZP000148A_seq_ST25.txt

ctgatgggtcc catccacgtt gaagcgtgt cttgccccca gtcttggatc aggcacctga	1260
ctctaaatga tgacgtcacc ccaggatttg ttgcctaac atcccttcgc attgagccga	1320
acacagagcc tactactttc cggatctttc ggtttggagc gcataagtgg tatggcgctg	1380
ccggcaaacg ggctcgtgct aagcgtgccg ctaaagggtga gaagaattca gctcccaccc	1440
ccaaggtcgc cctgccggtc cccacctatg gaattaccac ctactctcca ccgacagacg	1500
ggctcttggtg ttggcatgtc cttgccgcca taatgaaccg gatgataaat ggtgacttca	1560
cgtccccctc gactccgtac aacagaccag aggatgattg ggcttctgat tatgatcttg	1620
ctcaggcaat ccaatgtcta caactgcctg ctaccgtggc cggagtcgc gcctgtccca	1680
acgccaagta cctcataaaa ctcaacggag tccactggga ggtagagggt aggtcaggaa	1740
tggctcctcg ctctctttct cgtgaatgtg tggttggcgt ttgctctgaa ggttggttg	1800
caccgcctta tccagcagac gggctaccag aacgagcact cgaggccttg gcgtctgctt	1860
acaggttacc ctctgattgt gttagctctg gcattgctga ctttcttgct aaccacctc	1920
ctcaggaatt ctggaccctc gacaaaatgt tgacctcccc gtcaccagag cgggccggct	1980
tctctagttt gtataaatta ctattagagg ttgttccgca aaaatgcggg gccacggaag	2040
gggctttcgt ctatgctgtt gagagaatgt tgaaggattg tccgagctcc aaacaggcca	2100
tggcccttct ggcaaaaatt aaagtcccat cctcaaaggc cccgtctgtg tcccttgacg	2160
agtgtttccc tacggatgtt tcagccgact tcgagccagc atctcaggaa aggcccaaaa	2220
gttccgggtg tgctgttgct ctgtgttcac cggaagtaaa agagttcgaa gaagcagccc	2280
cagaagaagt tcaagagagt ggccacaagg tcgcccgtc tgcatthgtt gccgagggtc	2340
ctaacaatga acaggtaccg atggctgccg gcgagcaact gaagcccggg ggtcgcgttt	2400
tggcggtcgg gaatgctcat gaagggtgtt tggcctcaac tagttcgacc aacctgatag	2460
gcgggaactt ccccccttca gactccatga aagagagcat gctcaatagc tgggaagacg	2520
aaccactgga tttgtcccaa ccggcaccag ctgttacaat gacccttggt agagagcaaa	2580
cacccgacaa cctgggtcct gatgccggtg ctttccccgt caccgttcga aaatttgtcc	2640
cgacagggcc tacactccgt catgttgagc actgcggcac ggagtcaggc gacagcagtt	2700
cgcccttgga tctgtcttat gcgcaaacc tggaccagcc tttaaata tcttggccg	2760

ZP000148A_seq_ST25.txt

cttggccagt gagggccacc gcgtctgacc ctggctgggt ccacggtagg cgtgagccta	2820
tctttgtgaa gcctcgagat gctttctctg acggcgattc agcccttcag ctcggggagc	2880
tgtctgaatc cggctccgtc atcgagtttg accggacaag aaatgctccg gcggctgacg	2940
cccctgttga cttgacggct tcgaacaagg ccctctctgt ggttgatcct ttcgaatttg	3000
ccgaactcaa gcgcccgcgt ttttccgcac aagccttaat tgaccgaggc ggtccacttg	3060
ccgacgtcca tgcaaaaata aagaaccggg tatatgaaca gtgcctccag gcttgtgagc	3120
ctggcagtcg cgcaacccca gccaccaaga agtggctcga taaaatgtgg gatagggtgg	3180
acatgaagac ttggcgctgt acctcgcagt tccaagctgg tcgcattctt gcgtccccta	3240
aattcctccc tgacatgatt caggacacac cacctcctgt ccccttcaaa accggttgga	3300
ccaacacttg accaaattgt cccttcgccc acgggtaccc agcaagaaga tgtcacttcc	3360
tccgatgggc catctcatgc gccggatcct cctagtcgaa tgagcacgag tgggagttgg	3420
aagggccttg tgctctctgg taccctgtct gcgggggtcca ttagtcagca tttcatgaca	3480
tgggtttttg aggttttctc ccatctccca gcttttgac tcacactttt ctcgccgagg	3540
ggctctatgg cttcaggtga ttggatgttt gcagggtgtt ttttacttgc tctcctgctc	3600
tgtcgttctt acccagtatt cgggtgcctt cccttattgg gtgtcctttc tggctctgtg	3660
cggcgcgttc gtctgggggt ttttggttct tggatggctt tcgctgtatt tttattctcg	3720
actccatcca acccagtcgg ttcttcttgt gaccacgatt cgccggagtg tcatgctgag	3780
cttttggtc ttgagcagcg ccaactttgg gaacctgtgc gcggccttgt ggtcggcccc	3840
tcgggcctct tatgtgtcat tcttggcaag ctactcgggtg ggtcacgtta tctctggcat	3900
gttttcttac gtttatgcat gcttgcagat ttggcccttt ctcttgttta tgtggtgtcc	3960
caggggcgtt gtcacaagtg ttggggaaag tgtataagaa cagctccggc ggaggtggct	4020
ctcaatgtgt tccctttctc gcgcgctacc cgtagctctc ttgtgtcctt gtgcgatcgg	4080
ttccaagcgc caaaaggggt tgatcctgtg tacttggcaa cgggttggcg cgggtgttgg	4140
tgtggtgaga gtcccattca tcaaccgcac caaaaacca tagcttatgc taatctggat	4200
gaaaagaaaa tatctgcca aacggtggtt gctgtccac acgatcccag tcaggccatc	4260
aagtcctga aagttttgca ggcgggagga gccatcgtgg accagcccac acctgaggtc	4320

ZP000148A_seq_ST25.txt

gtccgtgtat ccgaaatccc cttctcagcc ccatttttcc caaaagttcc agtcaaccca	4380
gattgtaagg ttgtgggtgga ttcggacact tttgtggctg cggttcgctg cggctactcg	4440
acagcacaac tgggtcttagg ccagggcaac tttgccaagt taaatcagat tcccctcagg	4500
agctctatct ccaccaaagc gattggcgagg gcctcttaca cccttgctgt ggctcaagtt	4560
tctgtgtgga ctcttggtca cttcatcctc ggtcttttgt tcacgtcacc ccaagtgtgt	4620
ggccgaggaa cctctgactc atggtgttca aatccttttt cataccctac ctatggcccc	4680
ggggttggtg gtcctctctg actttgtgtg tctgccgacg gggtcactct accattgttc	4740
tcagccgtgg ctcaactctc cggtagagag gtggggattt ttatttttgt actcgtctcc	4800
ttgatggctt tggcccaccg catggccctt aaggcagaca tgttgatggg ctttctggct	4860
ttttgtgctt acgcctggcc catgagctcc tggttgattt gcttctttcc tacactcttg	4920
aagtgggtta ccctccacc ctttactatg ctttgggtgc actcattctt ggtgttttgt	4980
ctgccagcag ccggcatcct ctactaggg ataactggcc ttctttgggc ggttggccgc	5040
tttactcagg ttgccggaat tattacacct tatgacatcc accagtacac ctctgggcca	5100
cgtggcgctg cagctgtggc cacagcccca gaaggcactt atatggccgc cgtccggaga	5160
gctgcttta ctgggcgaac tttaatcttc acccgtctg cagttggatc ctttctcgaa	5220
ggagctttca ggactcataa accttgctt aacaccgtaa atgttgtagg ctcttcctt	5280
ggttccggcg gggttttcac tattgacggc agaaaaattg ttgtcactgc tgcccatgtg	5340
ttgaacggcg acacagctag agtcaccggc gactcctaca accgcatgca tactttcaag	5400
accaatggtg attatgcctg gtcccatgct gatgactggc ggggcgttgc ccctgcggtc	5460
aaggttgca aggggtaccg cggctgtgcc tactggcaaa catcaactgg cgtcgaacct	5520
ggtgttattg gggaagggtt cgccttctgt ttcaccacct gtggcgattc ggggtcacc	5580
gtcatctcag aatccggtga tctcattgga atccataccg gttcaaataa acttggttct	5640
ggtcttgatga caaccctga aggggagaca tgtaccatca aagaaaccaa gctctctgac	5700
ctctccagac atttcgcagg cccaagcgtt cctcttgggg atattaaatt gaggccagcc	5760
atcatccctg atataacatc cattccgagt gacttggcat cgctcctatc ctccgtccct	5820
gtagtggaag gcggcctctc gaccgttcaa cttttgtgtg tctttttcct actttggcg	5880

ZP000148A_seq_ST25.txt

atgatgggcc atgcctggac tcccattgtt gccgtgggtt tctttttact gaatgaaatt	5940
cttccagcag ttttgggtccg agccgtgttt tcttttgcac tttttgtgct tgcattgggcc	6000
accccctggg ctgcacaggt gttgatgatt agactcctca cggcatctct caaccgcaat	6060
aagctttctc tggcgttcta cgcactcggg ggggtcgtcg gtttggccgc tgaatcggg	6120
acttttgctg gcaaattgcc tgaattgtct caaacccttt cgacatactg cttcttacct	6180
agggtccttg ctatgaccag ttgtgttccc accatcatca ttgggtggact ccatgccctc	6240
ggtgtaattc tgtggttggtt caaataccgg tgcctccaca acatgctcgt tggatgagg	6300
agtttttcaa gcgccttctt cctacgggtat tttgcagagg gtaatctcag aaaagggtgtt	6360
tcgcagtcct gtggcatgaa taatgagtcg ctgacggctg ctttggcttg caagttgtca	6420
cgggctgacc ttgatttttt gtccagctta acgaacttca agtgctttgt atctgcttcg	6480
aatatgaaaa atgctgccgg ccagttacatt gaagcggcgt atgccaaggc cctgcgcaa	6540
gagttggcgt ctctagttca gattgacaaa atgaaaggag ttttgtccaa actcagggcc	6600
tttgctgaaa cagctactcc gtcccttgac atagggtgacg tgattgttct gcttgggcaa	6660
catccacacg gatctatcct tgatattaat gtggggactg agaggaaaac tgtgtccgtg	6720
caagagaccc ggagcctagg cggttccaaa ttcagtgttt gtactgtcgt gtctaacaca	6780
cccgtggacg ccttaaccgg catcccactc cagacaccaa cccctctttt tgagaatggg	6840
ccgcgtcatc gcagcgaaga agacgatctt aaagtcgaga ggatgaagaa aactgtgtg	6900
cccctcggct tccacaacat caatggcaaa gtttactgca agatttggga caagtccacc	6960
ggtgacacct tttaacacga tgattcccgg tatacccacg accatgctct tcaggacagg	7020
tcagccgact acagagacag ggactatgag ggtgtgcaaa ccgccccca acagggattt	7080
gatccaaagt ctgaaacccc tgtcggcact gttgtgatcg gcggtattac gtataacagg	7140
tatctgacta agggtaagga ggttcttggtt cccaagcccg acaattgcct tgaagctgcc	7200
aagctgtctc ttgagcaagc tctcgtcggg atgggcaaaa cttgcgacct tacagctgcc	7260
gaggtggaaa agttaaaagc catcatcagt caactccaag gtttgaccac tgaacaggct	7320
ttaaactgct agccgctagc ggcttgaccc gctgtggccg cggcggctta gttgttactg	7380
aaacggcggg aaaaattgta agataccaca gcagaacttt caccttgggc ctttggacc	7440

ZP000148A_seq_ST25.txt

taaaagtcgc ttctgaggtg gaggtgaaga gatcaactga gcagggccac gctgttgtgg	7500
caaacctatg ttctggtggt gtattgatga gacctcacc accgtccctt gttgacgttc	7560
ttctgaaacc cggacttgac acaacacccg gcattcaacc ggggcatggg gccgggaata	7620
tgggcgtgga cggttcatt tgggattttg aaaccgcacc cacaaaggca gagctcgagt	7680
tgtccaggca aataatccaa gcatgtgaag tcaggcgcgg ggatgccccg aacctccaac	7740
tcccttaciaa gctctgtcca gttagggggg atcctgagcg gcataaaggc cgccttatca	7800
ataccaggtt tggagatttg ctttacaaga ctctcaaga caccaagtcc gcaatccacg	7860
cggcttgttg cctgcacccc aacggggctc ccgtgtctga tggtaaattc acattaggca	7920
ccactcttca acacggtttc gagctttatg tccctactgt gccctatagt gtcattggagt	7980
accttgattc acgccctgac acccccttta tgtgtaccaa acatgggtacc cccgaggctg	8040
ctgcagagga ccttcgaaaa tatgacttat ccaccaagg gtttgtcctg cctgggggtcc	8100
tacgtctagt acgcagattc atctttggcc atattggtaa ggcaccgcca ctgttcctcc	8160
catcaactta tcccgccaag aactctatgg cagggatcaa tggccagagg ttcccaaciaa	8220
aagacgttca gagcatacct gaaattgatg aaatgtgtgc ccgcgctgtc aaagagaatt	8280
ggcaaaactgt gacgccttgc accctcaaaa aacagtattg ttccaagcct aaaaccagga	8340
ccatccttgg taccaaciaa ttcattgcct tggctcacag gtcggcactc agcgggtgtca	8400
ctcaggcggt catgaaaaaa gcctggaagt ccccgatcgc cttggggaaa aaciaattta	8460
aggagttgca ctgcactgtc gccggtaggt gcctcgaggc cgacttggcc tcttgtgatc	8520
gcagcacccc ggccatcgtg aggtgggtttg ttgccaacct cctgtacgaa cttgcgggtt	8580
gtgaagagta cttgccaagc tatgtgctca attgttgcca tgacctgggt gcaacgcaaa	8640
atggcgcctt caaaaaacgt ggtggcctgt catctggaga ccccgttacc agtgtgtcca	8700
acacagtgtt ttcactggtg atttatgctc agcacatggt gctgtcggct ttgaagatgg	8760
gtcatgaaat cggcctcaag ttcctcgagg aacagcttaa gttcgaggac cttctcgaaa	8820
tccagcctat gttggtatat tctgatgacc ttgtattgta tgctgaaaga cccaccttcc	8880
ccaattacca ttggtgggtt gagcaccttg acctgatgct gggtttttaag acagacccaa	8940
aaaagactgt cataactgac aaaccagct ttcttggtg tagaatcgaa gcggggcgac	9000

ZP000148A_seq_ST25.txt

aactggttcc cagtcgcgac cgcattctgg ctgctcttgc atatcacatg aaggcgcaga	9060
acgcctcaga gtattatgcg tctgctgccg cgattctgat ggactcgtgt gcttgcatgt	9120
accacgatcc tgagtggatg gaggacctca tctgcggcat tgctcgatgc gcccgccagg	9180
atggctacag tttcccaggc ccgccgttct tcatgtccat gtgggagaag ctgaaaagtc	9240
acaatgaagg caagaaattc cgccactgtg gcatttgtga cgccaaggcc gatcatgcgt	9300
ccgcctgtgg gcttgattta tgtctgttcc actcgcactt tcatcaacac tgccctgtta	9360
ctctgagctg cggccatcat gccggttcaa aagaatgccc acagtgtcag tcacctgttg	9420
gagctggtaa atccccctt gatgcagtgc tgaacaaat cccgtacaaa cccctcgtc	9480
ctgtcatcat gaaggtggac aataaaacaa cgacccttga cccgggaagg tatcagtccc	9540
gtcgaggtct tgttgctgtc aagaggggta ttgcaggcaa tgaggttgat ctgctgatg	9600
gagactatca ggtggtgccc cttttgcaa cctgcaaaga cataaacatg gtgaaggtgg	9660
ctgtcaatgt gctactcagc aagttcatag tggggccgcc aggttccggc aagacgacct	9720
ggctactgag tcaagttcag gatgatgatg tcatctatac acctacccat cagacctagt	9780
tcgacatagt caatgccctc aaagtctgta ggtattccat cccagtggct tcagggtccc	9840
ctttcccacc gcccgcaga tctggaccgt gggttaggct cgttgctagc gggcacatcc	9900
ctggccgaat atcatacctt gacgaggccg gatattgcaa tcatctggat attctcagac	9960
tgctttctaa gacacctctt gtgtgtttgg gtgaccttca gcaacttcac cctgtcggct	10020
ttgactctta ctgttatgtt tttgatcaca tgcctcataa gcagctgacc actatttata	10080
gatttggtccc caatatctgt tctgccattc aaccttgta cagggaacaa ctcgaatcta	10140
aggccaggaa cactaggggtg gtttttactg cccggcccgt ggcttttggt caggtgttga	10200
cgccatatca taaagaccgc accggctcag ctataacat agattcatcc caggagacca	10260
cctttgatgt tgtgacgtg catttgccgt cgccagattc cctgaacaaa tcccgggctc	10320
tttagctat cactcgggca aggcattgggt tgttcatcta tgacctcat aaccaactcc	10380
ggaagttttt caacttaaca cctgagcgca ctgattgcaa cctcgtgttc aaccgcgggg	10440
acgagttggt agttttgaat gcagataatg cagtcacgac tgtggctaag gttctggagg	10500
cgggcccgtc tcggtttcga gtatcagatc caaggtgcaa gtctctttta gccgcttgct	10560

ZP000148A_seq_ST25.txt

cggccagtct ggaaggaggc tgcatgccac tgccgcaagt ggcacataat ctgggggtttt	10620
acttctctcc agatagtcca gcatttgcac ctctgccaaa agagctggca ccacattggc	10680
cggttgttac tagtcagaac aaccaggcat ggcccgaccg acttgctgct agtatgcggc	10740
cagttgatgc ccgctacagc aagcctatgg tcggtgcagg gtatgtgggtt gggccatcca	10800
ctttccttgg cactcctggg gtgggtgtcat actatctcac gctgtacatc aggggtgagc	10860
cccaggcctt gccggagaca ctctgtctcaa cgggacgtat agctactgat tgtcgagagt	10920
atctcgacgc agctgaggaa gaagcagcta aagaactccc tcacgcattc attggcgatg	10980
tcaaaggtac tacagtgggg ggggtgtcacc acattacgtc aaaatacctt cccaggctcct	11040
tgcccaagga ctccgttgcc gtggctcggag tgagttcgcc cggcaaggct gccaaagccg	11100
tgtgtactct caccgatgtg tatcttcccg agctccggcc atatttgcaa ccggaaacag	11160
catcgaaatg ctggaaactt aaactagact tcagggatgt cagactgatg gtctggaaaag	11220
gagccaccgc atattttcaa ctggagggggc tcacatgggtc agcgctgccc gactatgcca	11280
ggttcattca gctacaaaaa gatgccgttg tgtacattga tccgtgcata ggaccggcaa	11340
cagccaaccg taaggttgtg agaactacag attggcgagc tgacctggca gtgacaccgt	11400
acgactacgg tgctcagagc attttgacta cagcctgggtt cgaggacctt gggccgcagt	11460
ggaagatfff agggttgcaa cccttcaagc gggcatttgg ctttgaaaac actgaggatt	11520
gggcgatcct tgcacgtcgc atgaacgacg gcaaggacta cactgactat aactggaatt	11580
gtgttcgaca acgcccacat gccatctacg gacgtgcccg tgaccatacg tatcactttg	11640
cccctggcac tgaactgcaa gtagagctag gtaaacctcg gctaccgcct gagcaagtac	11700
cgtgaatcta gagtgatgca atgggggtcac tgttgagtaa aatcagtcaa ctgttcgtgg	11760
atgccttcac tgagttcctt gttagtgtgg ttgatattgt catcttcctt gccatactgt	11820
ttgggttcac cgttgcaggg tggttactgg tctttttttt cagagtgggtt tgctccgcgc	11880
ttctccgttc gcgctctgcc attcactctt ccgaactatc gaaggctcta tgagggcttg	11940
ctacctaatt gcagaccgga tgttccacaa ttgcattca agcaccatt gggtatgttt	12000
tggcacatgc gaggttccca ctttaattgac gaaatggtct ctcgccgcat ttatcagacc	12060
atggaacatt caggtcaagc ggcctggaag caggtgggta gtgaagctac tctcacaaga	12120

ZP000148A_seq_ST25.txt

ctgtcaaagc tcgacatagt tctccacttc caacacctgg ccgcaataga ggcggattct	12180
tgccgcttcc tcagctcacg acttgtgatg ctgaaaaatc ttgctgttgg caatatgagc	12240
ctacagtaca acaccacgtt ggaccgcgtt gagctcatct tcccaacacc aggtacgagg	12300
cccaaattga ccgatttttag acaatggctc gtcagtgtgc acgcttctat tttttcctct	12360
gtggcctcat cagttacctt gttcatagtg ctttggcttc gaattccagc tctacgctat	12420
gtttttgggt tccattggcc cacggcaaca catcattcga gctaaccatc aattactcta	12480
tatgtaagcc ctgtctcacc agtcaagcgg ctcgacaaag gctcgaaccc ggtcgcaaca	12540
tgtggtgcaa aatagggcac accacgtgtg aggagcgtga ccatgatgag ttgtcaatga	12600
ccattccgtc cgggtacgat aacctcaaac ttgagggtta ttacgcttgg ctggcttttt	12660
tgtccttttc ttacgcagcc caatttcac cagagttgtt cggaataggg aatgtgtcac	12720
gcgctctcgt ggataaacgg caccagttca tctgtgccga gcacgacgga caaaattcaa	12780
ccgtatccac cgaacataat atttccgcat tgtatgcggc gtactaccac caccaggtag	12840
acggggggcaa ttggttccac ctggaatggc tgcggccttt cttttcctcc tggctagtac	12900
tcaatatttc atggtttctg aggcgttcgc ctgcaagccc tgtttctcga cgcatttatc	12960
agatattgag accaacacga ccgcggctgc cggtttcatg gtccttcagg acatcaactg	13020
tttccacagt ggctcagagg cacaaacgac tgggtcccatc agaaagtcgt cccaatgccg	13080
tgaagccgtc ggcactcccc agtacatcaa gataacggcc aatctgaccg acgaatcata	13140
cttgtaaac gcggacttgc tgatgctttc tgcgtgcctt ttctacgcct cagagatgag	13200
cgagaaaggc tttaaagtca tttttgggaa tgtctctggc gttgtctccg cttgtgtcaa	13260
ttttacagat tatgtggccc atctgaccca acatacccag cagcatcatt tggtaaccaa	13320
tcacattcgg ttgctacatt tcctgacacc atcagcgatg aggtgggcta caaccattgc	13380
ttgtttgttc gccattctct tggcgatatg agatgttctc acaaatcggg gtgtttcttg	13440
actccgcact cttgcttttg gtggtttttt ttgctgtgta ccggcttgtc ctggtccttt	13500
gccgatggca acggcgacag ctcgacatac caatatatat ataacttgac gatatgagag	13560
ctgaatggga ccgaatggct gtctgaccat ttttaattggg cagtcgagac ttttgtgctc	13620
taccagtggt cgactcatat cctctcactg ggtttcctca cgacaagtca tttccttgat	13680

ZP000148A_seq_ST25.txt

gcgttcggtc ttggagctgt gtcaattaca gggttttgtg gcgggcggta cgtgctcagc	13740
agcgtgtacg gcgcttgtgc actagcagcg ctctgtatgtt ttgttatccg tgctgcaaaa	13800
aattgtatgg cttgccgcta tgctcgtacc cggttttacca acttcattgt ggatgaccgg	13860
gggagaatcc atcgggtggag gtctccaata gtgggtggaaa aattaggtaa agctgacgtc	13920
ggcggcgacc ttgtcaccat caaacatgtc atcctcgaag gagtcaaagc tcaacccttg	13980
acgaggactt cggccgagca atgggaggcc tagataatth ctgcaacgat cccaccgccg	14040
cacaaaagct tgtgctagcc tttagcatca cgtatacacc catcatgata tacgccctta	14100
aggtgtcacg cggccgactc ttgggggttgt tgcacatctt gatatttctg aactgttctt	14160
tcacgttcgg atacatgaca tatatgcatt ttgaatccac caaccgtgtc gcgcttacca	14220
tggggggcgt tgtcgccctt ctgtggggcg tttatagttt catagagtca tggaagttha	14280
tcacttccag atgcagattg tgttgccctag gccggcgata cattctggcc cctgcccacc	14340
acgtagaaag tgctgcaggc ctccatccga tcccagcgtc tggtaaccaa gcatacgtg	14400
tgagaaagcc cggactaaca tcagtgaacg gcactctggg accaggactt cgaggcctcg	14460
tgctgggcgg caaacgagct gttaaagcag gagtgggttaa cctcgtcaag tatggccggt	14520
aaaaaccaga gccagaagaa aaagaaaaat ccagctccaa tggggaatgg ccagtcagtc	14580
aatcaactgt gccagctgct gggcacaatg ataaagtccc agcgccagcg acccagggga	14640
ggacaggcta aaaagaaaaa gcctgagaag ccacatttcc ccctggctgc tgaagatgac	14700
gtccggcacc atctcaccca gaccgagcgc tccctttgct tgcaatcgat ccagacggct	14760
tttaatcaag gcgcaggaac tgcgtcgctt tcatccagcg ggaagatcgg ttttcaggtt	14820
gagtttatgc taccggttgc tcatacagtg cgcctgattc gcgtgacttc tacatccgcc	14880
ggtcaggatg caaattaatt tgatagtcag gtgaatggcc gcgattggcg tgtggcctct	14940
gagtcaccta ttcaattagg gcgatcacat gggggtcaga cttaattagg caggaaccat	15000
gtgaccgaaa tttcctagga agaatcgggc tagtgataat gccggcctga agcgactggg	15060
ggcgcagtgg gacagaaaaat tgagtgaac cc	15092

<210> 5

<211> 15092

<212> DNA

<213> Artificial Sequence

<220>

<223> PRRS virus isolate 96V198 - passage 49

<400> 5

atgatgtgta gggatttccc cctacatata cgacacttct agtgtttgtg tgccttggag	60
gcgtgggtat agccccgccc cacctcttgg cccctgttct agcccaacag gtatccttct	120
ctctcggggc gagcgcgccg cctgctgctc ccttgacgca ggaaggacct cccgagtatt	180
tccggagagc acctgcttta cgggatctcc accctttaac catgtctggg acgttctccc	240
ggtgcatgtg caccggtt gcccggttat tttggagcgc cggccaagtc tattgcacac	300
ggtgtctcag tgcacggtct cttctctctc cggagcttca ggacactgac ctgctgcaa	360
ttggcttggt ttacaagcct aaagacaagc ttacttgga agtccctatc ggcatccctc	420
aggtggagtg tactccgtcc ggggtgctgtt ggctctcagc catcttcccc ttggcgcgca	480
tgacctccgg caatcacaac tttctccaac gacttgtgaa ggttgctgat gttttgtacc	540
gtgacggctg cttggcacct cgacaccttc gcgaactcca agtttacgag cgcggctgca	600
actggtaccc gatcacgggg cccgtgcccg ggatgggttt gtacgcgaac tccatgcacg	660
tgtccgacca accgttccct ggtgccgctt atgtgttaac gaactcacct ctgcctcagc	720
aggcttgtcg acagccgttt tgtccatttg aggaggttca ttccgacgta tacagggtgga	780
agaaattcgt gatttttgtg gattcctctt ctgacggccg atctcgcatg atgtggacac	840
caggatccga cgactcggct gccttagaag tattgccacc tgaactagaa tgtcgagtcg	900
aaatcctcgt tcggagtttt cctgcccacc accctgtcga catcaccaac tgggagctca	960
ctgagtcccc tgagcacggt ttttccttca gcacgtctca ttcttggtgt taccttgccc	1020
aagaccctga cgtgtttgat ggtaagtgtt ggctttcttg ctttttgggc ctgccgaccg	1080
aagtgtggcg tcatgaggag catctagcta acgccttcgg ttaccaaacc aagtggggcg	1140
tgcatggtaa gtacctccag cgcaggcttc aggtccgcgg catgcgtgct gtagttgatc	1200
ctgatggtcc catccacgtt gaagcgctgt cttgccccca gtcttggtac aggcacctga	1260
ctctaaatga tgacgtcacc ccaggatttg ttgcctaac atcccttcgc attgagccga	1320
acacagagcc tactactttc cggatctttc ggtttggagc gcataagtgg tatggcgctg	1380

ZP000148A_seq_ST25.txt

ccggcaaacg ggctcgtgct aagcgtgccg ctaaaggatga gaagaattca gctcccaccc	1440
ccaaggctgc cctgccggtc cccacctatg gaattaccac ctactctcca ccgacagacg	1500
ggctcttggtg ttggcatgtc cttgccgcca taatgaaccg gatgataaat ggtgacttca	1560
cgtccccctct gactccgtac aacagaccag aggatgattg ggcttctgat tatgatcttg	1620
ctcaggcaat ccaatgtcta caactgcctg ctaccgtggg ccggagtcgc gcctgtccca	1680
acgccaagta cctcataaaa ctcaacggag tccactggga ggtagagggtg aggtcaggaa	1740
tggctcctcg ctctctttct cgtgaatgtg tggttggcgt ttgctctgaa ggttggtgtg	1800
caccgcctta tccagcagac gggctaccag aacgagcact cgaggccttg gcgtctgctt	1860
acaggttacc ctctgattgt gttagctctg gcattgctga ctttcttgct aaccacctc	1920
ctcaggaatt ctggaccctc gacaaaatgt tgacctcccc gtcaccagag cgggccggct	1980
tctctagttt gtataaatta ctattagagg ttgttccgca aaaatgcggg gccacggaag	2040
gggctttcgt ctatgctgtt gagagaatgt tgaaggattg tccgagctcc aaacaggcca	2100
tggcccttct ggcaaaaatt aaagttccat cctcaaaggc cccgtctgtg tcccttgacg	2160
agtgtttccc tacggatgtt tcagccgact tcgagccagc atctcaggaa aggcccaaaa	2220
gttccgggtgc tgctgttgct ctgtgttcac cggaagtaaa agagttcgaa gaagcagccc	2280
cagaagaagt tcaagagagt ggccataagg tcgcccgtc tgcatthgtt gccgagggtc	2340
ctaacaatga acaggtaccg atggctgccg gcgagcaact gaagcccggg ggtcgcgttt	2400
tggcggtcgg gaatgctcat gaaggtgttc tggcctcaac tagttcgacc aacctgatag	2460
gcgggaactt cccccctca gactccatga aagagagcat gctcaatagc tgggaagacg	2520
aaccactgga tttgtcccaa ccggcaccag ctgttacaat gacccttggt agagagcaaa	2580
cacccgacaa cctgggtcct gatgccggtg cttccccgt caccgttcga aaatttgtcc	2640
cgacagggcc tacactccgt catgttgagc actgcggcac ggagtcaggc gacagcagtt	2700
cgcccttgga tctgtcttat gcgcaaacc tggaccagcc tttaaatacta tccttggccg	2760
cttggccagt gagggccacc gcgtctgacc ctggctgggt ccacggtagg cgtgagccta	2820
tctttgtgaa gcctcgagat gctttctctg acggcgattc agcccttcag ctcggggagc	2880
tgtctgaatc cggctccgtc atcgagtttg accggacaag aaatgctccg gcggtcgacg	2940

ZP000148A_seq_ST25.txt

cccctgttga cttgacggct tcgaacaagg ccctctctgt ggttgatcct ttcgaatttg	3000
ccgaactcaa gcgcccgcgt ttttccgcac aagccttaat tgaccgaggc ggtccacttg	3060
ccgacgtcca tgcaaaaata aagaaccggg tatatgaaca gtgcctccag gcttgtgagc	3120
ctggcagtcg cgcaacccca gccaccaaga agtggctcga taaaatgtgg gatagggtgg	3180
acatgaagac ttggcgctgt acctcgcagt tccaagctgg tcgcattctt gcgtccctta	3240
aattcctccc tgacatgatt caggacacac cacctcctgt tcctaggaag aatcgggcta	3300
gtgataatgc cggcctgaag cgactgggtg cgcagtggga cagaaaattg agtgcaaccc	3360
ccccttcaaa accggttgga ccaacacttg accaaattgt cccttcgccc acgggtaccc	3420
agcaagaaga tgtcacttcc tccgatgggc catctcatgc gccggatcct cctagtcgaa	3480
tgagcacgag tgggagttgg aagggccttg tgctctctgg taccctctc gcggggtcca	3540
ttagtcagca tttcatgaca tgggtttttg aggttttctc ccatctcca gcttttgac	3600
tcacactttt ctgccgagg ggctctatgg cttcaggtga ttggatgttt gcaggtgttg	3660
ttttacttgc tctcctgctc tgtcgttctt acccagtatt cgggtgcctt cccttattgg	3720
gtgtcctttc tggctctgtg cggcgcgctt gtctgggggt ttttggttct tggatggctt	3780
tcgctgtatt tttattctcg actccatcca acccagtcgg ttcttcttgt gaccacgatt	3840
cgccggagtg tcatgctgag cttttggctc ttgagcagcg ccaactttgg gaacctgtgc	3900
gcggccttgt ggtcggcccc tcgggcctct tatgtgtcat tcttggcaag ctactcggtg	3960
ggtcacgtta tctctggcat gttttcttac gtttatgcat gcttgcagat ttggcccttt	4020
ctcttgttta tgtggtgtcc caggggcgtt gtcacaagtg ttggggaaag tgtataagaa	4080
cagctccggc ggaggtggct ctcaatgtgt tccctttctc gcgcgctacc cgtagctctc	4140
ttgtgtcctt gtgcgatcgg ttccaagcgc caaaaggggt tgatcctgtg tacttggcaa	4200
cgggttggcg cgggtgttgg tgtggtgaga gtcccattca tcaaccgcac caaaaacca	4260
tagcttatgc taatctggat gaaaagaaaa tatctgcca aacggtggtt gctgtccac	4320
acgatcccag tcaggccatc aagtcctga aagttttgca ggcgaggga gccatcgtgg	4380
accagcccac acctgaggtc gtccgtgtat ccgaaatccc cttctcagcc ccatttttcc	4440
caaaagttcc agtcaacca gattgtaagg ttgtggtgga ttcggacact tttgtggctg	4500

ZP000148A_seq_ST25.txt

cggttcgctg cggctactcg acagcacaac tggctcttagg ccagggcaac tttgccaagt	4560
taaatcagat tcccctcagg agctctatct ccaccaaagc gattggcggg gcctcttaca	4620
cccttgctgt ggctcaagtt tctgtgtgga ctcttgttca cttcatcctc ggtctttggt	4680
tcacgtcacc ccaagtgtgt ggccgaggaa cctctgactc atgggtgttca aatccttttt	4740
cataccctac ctatggcccc ggggttgtgt gctcctctcg acttttgttg tctgccgacg	4800
gggtcactct accattgttc tcagccgtgg ctcaactctc cggtagagag gtggggattt	4860
ttattttggt actcgtctcc ttgatggctt tggcccaccg catggccctt aaggcagaca	4920
tgttgatggg ctttctggct ttttgtgctt acgcctggcc catgagctcc tggttgattt	4980
gcttctttcc tacactcttg aagtgggtta ccctccaccc tcttactatg ctttgggtgc	5040
actcattctt ggtgttttgt ctgccagcag ccggcatcct ctactaggg ataactggcc	5100
ttctttgggc ggttgccgc tttactcagg ttgccggaat tattacacct tatgacatcc	5160
accagtacac ctctgggcca cgtggcgctg cagctgtggc cacagcccca gaaggcactt	5220
atatggccgc cgtccggaga gctgctttaa ctgggcgaac tttaatcttc acccgtctg	5280
cagttggatc ctttctcgaa ggagctttca ggactcataa accttgctctt aacaccgtaa	5340
atgtttagg ctcttcctt ggttccggcg gggttttcac tattgacggc agaaaaattg	5400
ttgtcactgc tgcccatgtg ttgaacggcg acacagctag agtcaccggc gactcctaca	5460
accgcatgca tactttcaag accaatggtg attatgcctg gtcccatgct gatgactggc	5520
ggggcgttgc ccctgcggtc aaggttgcga aggggtaccg cggtcgtgcc tactggcaaa	5580
catcaactgg cgtcgaaccc ggtgttattg gggaagggtt cgccttctgt ttcaccacct	5640
gtggcgattc ggggtcacc gtcactcag aatccggtga tctcattgga atccataccg	5700
gttcaaataa acttggttct ggtcttgta caaccctga aggggagaca tgtaccatca	5760
aagaaaccaa gctctctgac ctctccagac atttcgcagg cccaagcgtt cctcttgggg	5820
atattaaatt gagtccagcc atcatccctg atataacatc cattccgagt gacttggcat	5880
cgctcctatc ctccgtccct gtagtggaag gcggcctctc gaccgttcaa cttttgtgtg	5940
tctttttcct actttggcgc atgatgggcc atgcctggac tcccattggt gccgtgggtt	6000
tctttttact gaatgaaatt cttccagcag ttttgggtccg agccgtgttt tcttttgcac	6060

ZP000148A_seq_ST25.txt

tttttgtgct tgcattgggcc accccctggg ctgcacaggt gttgatgatt agactcctca	6120
cggcatctct caaccgcaat aagctttctc tggcgttcta cgcactcggg ggggtcgtcg	6180
gtttggccgc tgaaatcggg acttttgctg gcaaattgcc tgaattgtct caaacctttt	6240
cgacatactg cttcttacct agggtccttg ctatgaccag ttgtgttccc accatcatca	6300
ttggtggact ccatgccctc ggtgtaattc tgtggttggt caaataccgg tgcctccaca	6360
acatgctcgt tggatgatggg agtttttcaa gcgccttctt cctacgggat tttgcagagg	6420
gtaatctcag aaaagggtgtt tcgcagtcct gtggcatgaa taacgagtcc ctgacggctg	6480
ctttggcttg caagttgtca cgggctgacc ttgatttttt gtccagctta acgaacttca	6540
agtgccttgt atctgcttcg aatatgaaaa atgctgccgg ccagtacatt gaagcggcgt	6600
atgccaaggc cctgcgcaa gagttggcgt ctctagttca gattgacaaa atgaaaggag	6660
ttttgtccaa actcgaggcc tttgctgaaa cagctactcc gtcccttgac ataggtgacg	6720
tgattgttct gcttgggcaa catccacacg gatctatcct tgatattaat gtggggactg	6780
agaggaaaac tgtgtccgtg caagagaccc ggagcctagg cggttccaaa ttcagtgttt	6840
gtactgtcgt gtctaacaca cccgtggacg ccttaaccgg catcccactc cagacaccaa	6900
cccctctttt tgagaatggg ccgcgtcatc gcagcgaaga agacgatctt aaagtcgaga	6960
ggatgaagaa acactgtgtg cccctcggct tccacaacat caatggcaaa gtttactgca	7020
agatttggga caagtccacc ggtgacacct ttacacgga tgattcccgg tataccacg	7080
accatgctct tcaggacagg tcagccgact acagagacag ggactatgag ggtgtgcaaa	7140
ccgccccca acagggattt gatccaaagt ctgaaacccc tgtcggcact gttgtgatcg	7200
gcggtattac gtataacagg tatctgacta agggtaagga ggcttctggt cccaagccc	7260
acaattgcct tgaagctgcc aagctgtctc ttgagcaagc tctcgctggg atgggcaaaa	7320
cttgcgacct tacagctgcc gaggtggaaa agttaaagcg catcatcagt caactccaag	7380
gtttgaccac tgaacaggct ttaaactgct agccgctagc ggcttgaccc gctgtggccg	7440
cggcggctta gttgttactg aaacggcggg aaaaattgta agataccaca gcagaacttt	7500
caccttgggc ctttggacc taaaagtcgc ttctgaggtg gaggtgaaga gatcaactga	7560
gcagggccac gctgttgtgg caaacctatg ttctgggtgt gtattgatga gacctaccc	7620

ZP000148A_seq_ST25.txt

accgtccctt gttgacgttc ttctgaaacc cggacttgac acaacacccg gcattcaacc	7680
ggggcatggg gccgggaata tgggcgtgga cggttccatt tgggattttg aaaccgcacc	7740
cacaaaggca gagctcgagt tgtccaggca aataatccaa gcatgtgaag tcaggcgcgg	7800
ggatgccccg aacctccaac tcccttaciaa gctctgtcca gttagggggg atcctgagcg	7860
gcataaaggc cgccttatca ataccagggt tggagatttg cttacaaga ctctcaaga	7920
caccaagtcc gcaatccacg cggcttggtt cctgcacccc aacggggctc ccgtgtctga	7980
tggtaaatcc acattaggca ccactcttca acacggtttc gagctttatg tccctactgt	8040
gccctatagt gtcattggagt accttgattc acgccctgac acccccttta tgtgtacca	8100
acatgggtacc cccgaggctg ctgcagagga ccttcgaaaa tatgacttat ccaccaagg	8160
gtttgtcctg cctgggggtc tacgtctagt acgcagattc atctttggcc atattggtaa	8220
ggcaccgcca ctgttcctcc catcaactta tcccgccaag aactctatgg cagggatcaa	8280
tggccagagg ttcccaacaa aagacgttca gagcatacct gaaattgatg aaatgtgtgc	8340
ccgcgctgtc aaagagaatt ggcaaaactgt gacgccttgc accctcaaaa aacagtattg	8400
ttccaagcct aaaaccagga ccactccttg taccaacaac ttcatcgctt tggctcacag	8460
gtcggcactc agcgggtgtc ctgaggcgtt catgaaaaaa gcctggaagt ccccgatcgc	8520
cttggggaaa aacaaattta aggagttgca ctgcactgtc gccggtaggt gcctcgaggc	8580
cgacttggcc tcttgatgac gcagcacccc ggccatcgtg aggtgggttg ttgccaacct	8640
cctgtacgaa cttgcgggtt gtgaagagta cttgccaagc tatgtgctca attgttgcca	8700
tgacctggtg gcaacgcaaa atggcgcctt caaaaaacgt ggtggcctgt catctggaga	8760
ccccgttacc agtgtgtcca acacagtgt ttcactggtg atttatgctc agcacatggt	8820
gctgtcggct ttgaagatgg gtcattgaaat cggcctcaag ttcctcgagg aacagcttaa	8880
gttcgaggac cttctcgaaa tccagcctat gttggtatat tctgatgacc ttgtattgta	8940
tgctgaaaga cccaccttcc ccaattacca ttggtgggtt gagcaccttg acctgatgct	9000
gggttttaag acagacccaa aaaagactgt cataactgac aaaccagct ttcttggtg	9060
tagaatcgaa gcggggcgac aactgggtcc cagtcgcgac cgcattctgg ctgctcttgc	9120
atatcacatg aaggcgcaga acgcctcaga gtattatgct tctgctgccg cgattctgat	9180

ZP000148A_seq_ST25.txt

ggactcgtgt gcttgcattg accacgatcc tgagtggat gaggacctca tctgcggcat	9240
tgctcgatgc gcccgccagg atggctacag tttcccaggc ccgccgttct tcatgtccat	9300
gtgggagaag ctgaaaagtc acaatgaagg caagaaattc cgccactgtg gcatttgtga	9360
cgccaaggcc gatcatgcgt ccgcctgtgg gcttgattta tgtctgttcc actcgcactt	9420
tcatcaacac tgccctgtta ctctgagctg cggccatcat gccggttcaa aagaatgccc	9480
acagtgtcag tcacctgttg gagctggtaa atccccctt gatgcagtgc tgaaacaaat	9540
cccgtaaaa cccctcgtc ctgtcatcat gaaggtggac aataaaacaa cgacccttga	9600
cccgggaagg tatcagtccc gtcgaggtct tgttgctgtc aagaggggta ttgcaggcaa	9660
tgaggttgat ctcgctgatg gagactatca ggtggtgccc cttttgccaa cctgcaaaga	9720
cataaacatg gtgaaggtgg ctgtcaatgt gctactcagc aagttcatag tggggccgcc	9780
aggttccggc aagacgacct ggctactgag tcaagttcag gatgatgatg tcatctatac	9840
acctacccat cagaccatgt tcgacatagt caatgccctc aaagtctgta ggtattccat	9900
cccagtggct tcagggtccc ctttcccacc gcccgccaga tctggaccgt gggtaggct	9960
cgttgctagc gggcacatcc ctggccgaat atcatacctt gacgaggccg gatattgcaa	10020
tcatctggat attctcagac tgctttctaa gacacctctt gtgtgtttgg gtgaccttca	10080
gcaacttcac cctgtcggct ttgactctta ctgttatgtt tttgatcaca tgcctcataa	10140
gcagctgacc actatttata gatttggccc caatatctgt tctgccattc aaccttgta	10200
cagggaaaaa ctcgaatcta aggccaggaa cactaggggtg gtttttactg cccggcccgt	10260
ggcttttggc caggtgttga cgccatatca taaagaccgc accggctcag ctataaccat	10320
agattcatcc caggagacca cttttgatgt tgtgacgtg catttgccgt cgccagattc	10380
cctgaacaaa tcccgggctc ttgtagctat cactcgggca aggcattgggt tgttcatcta	10440
tgacctcat aaccaactcc ggaagttttt caacttaaca cctgagcgca ctgattgcaa	10500
cctcgtgttc aaccgcgggg acgagttggc agttttgaat gcagataatg cagtcacgac	10560
tgtggctaag gttctggagg cgggcccgtc tcggtttcga gtatcagatc caaggtgcaa	10620
gtctctttta gccgcttgct cggccagtct ggaaggaggc tgcatgccac tgccgcaagt	10680
ggcacataat ctggggtttt acttctctcc agatagtcca gcatttgcac ctctgcaaaa	10740

ZP000148A_seq_ST25.txt

agagctggca ccacattggc cggttgttac tagtcagaac aaccaggcat ggccccgaccg	10800
acttgtcgct agtatgcggc cagttgatgc ccgctacagc aagcctatgg tcggtgcagg	10860
gtatgtggtt gggccatcca ctttccttgg cactcctggg gtgggtgtcat actatctcac	10920
gctgtacatc aggggtgagc cccaggcctt gccggagaca ctcgtctcaa cgggacgtat	10980
agctactgat tgtcgagagt atctcgacgc agctgaggaa gaagcagcta aagaactccc	11040
tcacgcattc attggcgatg tcaaaggtac tacagtgggg ggggtgtcacc acattacgtc	11100
aaaatacctt cccaggctct tgcccaagga ctccgttgcc gtgggtcggag tgagtctgcc	11160
cggcaaggct gccaaagccg tgtgtactct caccgatgtg tatcttcccg agctccggcc	11220
atatttgcaa ccggaaacag catcgaaatg ctggaaactt aaactagact tcagggatgt	11280
cagactgatg gtctggaaag gagccaccgc atatttccaa ctggaggggc tcacatggtc	11340
agcgctgccc gactatgcca gggttcattca gctacaaaaa gatgccgttg tgtacattga	11400
tccgtgcata ggaccggcaa cagccaaccg taaggttgtg agaactacag attggcgagc	11460
tgacctggca gtgacaccgt acgactacgg tgctcagagc attttgacta cagcctggtt	11520
cgaggacctt gggccgcagt ggaagatttt agggttgcaa cccttcaagc gggcatttgg	11580
ctttgaaaac actgaggatt gggcgatcct tgcacgtcgc atgaacgacg gcaaggacta	11640
cactgactat aactggaatt gtgttcgaca acgcccacat gccatctacg gacgtgccccg	11700
tgaccatacg tatcactttg cccctggcac tgaactgcaa gtagagctag gtaaacctcg	11760
gctaccgcct gagcaagtac cgtgaatcta gagtgatgca atgggggtcac tgtggagtaa	11820
aatcagtcaa ctgttcgtgg atgccttcac tgagttcctt gttagtgtgg ttgatattgt	11880
catcttcctt gccatactgt ttgggttcac cgttgcaggg tggttactgg tcttttttct	11940
cagagtgggt tgctccgcgc ttctccgttc gcgctctgcc attcactctt ccgaactatc	12000
gaaggtccta tgagggttg ctacctaatt gcagaccgga tgttccacaa ttcgcattca	12060
agcaccattt gggatatgtt ttggcacatgc gagtttccca cttaattgac gaaatgggtct	12120
ctcgccgcat ttatcagacc atggaacatt cagggtcaagc ggcttggaag cagggtggta	12180
gtgaagctac tctcacaaga ctgtcaaagc tcgacatagt tctccacttc caacacctgg	12240
ccgcaataga ggcggattct tgccgcttcc tcagctcacg acttgtgatg ctgaaaaatc	12300

ZP000148A_seq_ST25.txt

ttgctgttgg caatatgagc ctacagtaca acaccacgtt ggaccgcgtt gagctcatct	12360
tcccaacacc aggtacgagg cccaaattga ccgatttttag acaatggctc gtcagtgtgc	12420
acgcttctat tttttcctct gtggcctcat cagttacctt gttcatagtg ctttggcttc	12480
gaattccagc tctacgctat gtttttggtt tccattggcc cacggcaaca catcattcga	12540
gctaaccatc aattactcta tatgtaagcc ctgtctcacc agtcaagcgg ctcgacaaag	12600
gctcgaaccc ggtcgcaaca tgtggtgcaa aatagggcac accacgtgtg aggagcgtga	12660
ccatgatgag ttgtcaatga ccattccgtc cgggtacgat aacctcaaac ttgagggtta	12720
ttacgcttgg ctggcttttt tgtccttttc ttacgcagcc caatttcatc cagagttggt	12780
cggaataggg aatgtgtcac gcgtcttcgt ggataaacgg caccagttca tctgtgccga	12840
gcacgacgga caaaattcaa ccgtatccac cgaacataat atttccgcat tgtatgcggc	12900
gtactaccac caccaggtag acgggggcaa ttggttccac ctggaatggc tgcggccttt	12960
cttttcctcc tggctagtac tcaatatattc atggtttctg aggcgttcgc ctgcaagccc	13020
tgtttctcga cgcatttatc agatattgag accaacacga ccgcggctgc cggtttcatg	13080
gtccttcagg acatcaactg tttccacagt ggctcagagg cacaaacgac tgggtccatc	13140
agaaagtcgt cccaatgccg tgaagccgtc ggcaactcccc agtacatcaa gataacggcc	13200
aatctgaccg acgaatcata cttgtacaac gcggacttgc tgatgctttc tgcgtgcctt	13260
ttctacgcct cagagatgag cgagaaaggc tttaaagtca tttttgggaa tgtctctggc	13320
gttgtctccg ctttgttcaa ttttacagat tatgtggccc atctgacca acataccag	13380
cagcatcatt tggtaaccaa tcacattcgg ttgctacatt tcctgacacc atcagcgatg	13440
aggtgggcta caaccattgc ttgtttgttc gccattctct tggcgatatg agatgttctc	13500
acaaatcggg gtgtttcttg actccgcact cttgcttttg gtggtttttt ttgctgtgta	13560
ccggcttgtc ctggctcttt gccgatggca acggcgacag ctcgacatac caatatatat	13620
ataacttgac gatatgcgag ctgaatggga ccgaatggct gtctgaccat ttaattggg	13680
cagtcgagac ttttgtgctc taccagtggt cgactcatat cctctcactg ggtttcctca	13740
cgacaagtca tttccttgat gcgttcggtc ttggagctgt gtcaattaca gggttttgtg	13800
gcgggcggtg cgtgctcagc agcgtgtacg gcgcttgtgc actagcagcg ctcgtatgtt	13860

ZP000148A_seq_ST25.txt

ttgttatccg tgctgcaaaa aattgtatgg cttgccgcta tgctcgtaacc cggtttacca	13920
acttcattgt ggatgaccgg gggagaatcc atcgggtggag gtctccaata gtggtggaaa	13980
aattaggtaa agctgacgtc ggcggcgacc ttgtcaccat caaacatgtc atcctcgaag	14040
gagtcaaagc tcaacccttg acgaggactt cggccgagca atgggaggcc tagataat	14100
ctgcaacgat cccaccgccg cacaaaagct tgtgctagcc tttagcatca cgtatacacc	14160
catcatgata tacgccctta aggtgtcacg cggccgactc ttgggggttgt tgcacatctt	14220
gatatttctg aactgttctt tcacgttcgg atacatgaca tatatgcatt ttgaatccac	14280
caaccgtgtc gcgcttacca tggggggcgt tgtcgccctt ctgtggggcg tttatagttt	14340
catagagtca tggaagttta tcacttccag atgcagattg tgttgcctag gccggcgata	14400
cattctggcc cctgcccacc acgtagaaag tgctgcaggc ctccatccga tcccagcgtc	14460
tggtaaccaa gcatacgctg tgagaaagcc cggactaaca tcagtgaacg gcactctggt	14520
accaggactt cgaggcctcg tgctggggcg caaacgagct gttaaacgag gagtggttaa	14580
cctcgtcaag tatggccggt aaaaaccaga gccagaagaa aaagaaaaat ccagctccaa	14640
tggggaatgg ccagtcagtc aatcaactgt gccagctgct gggcacaatg ataaagtccc	14700
agcgccagcg acccagggga ggacaggcta aaaagaaaaa gcctgagaag ccacatttcc	14760
ccctggctgc tgaagatgac gtccggcacc atctcaccca gaccgagcgc tccctttgct	14820
tgcaatcgat ccagacggct tttaatcaag gcgcaggaac tgcgtcgctt tcatccagcg	14880
ggaagatcgg ttttcaggtt gagtttatgc taccggttgc tcatacagtg cgcctgattc	14940
gcgtgacttc tacatccgcc ggtcaggatg caaattaatt tgatagtcag gtgaatggcc	15000
gcgattggcg tgtggcctct gagtcaccta ttcaattagg gcgatcacat gggggtcaga	15060
cttaattagg caggaacat gtgaccgaaa tt	15092

<210> 6

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> oligonucleotide primer

<400> 6
gcaccacctc acccagac 18

<210> 7
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide primer

<400> 7
cagttcctgc gccttgat 18

<210> 8
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide primer

<400> 8
cctctgcttg caatcgatcc agac 24