VACCINES AGAINST AFRICAN SWINE FEVER VIRUS, AND METHODS OF USING SAME

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/950,194, filed December 19, 2019 which is hereby incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The present invention relates to a recombinant nucleotide sequence that encodes at least one African swine fever viral (ASFV) antigen, and functional fragments thereof. The invention also relates to a combination of multiple recombinant nucleotide sequences encoding ASFV antigens for generating immunity against African swine fever disease. The compositions of the invention provide improved methods for inducing immune responses, and for prophylactically and/or therapeutically immunizing individuals against African swine fever virus.

BACKGROUND OF THE INVENTION

African swine fever virus (ASFV) is a large, double-stranded DNA virus from the Asfarviridae family. ASFV is the causal agent for a hemorrhagic fever with high mortality in domestic pigs; some isolates can cause death of animals as quickly as a week after infection. ASFV persistently infects its natural hosts including warthogs, bushpigs, and soft ticks of the genus Ornithodoros, which likely act as a vector, without disease signs.

ASFV does not cause disease in humans; however, its impact can be felt through direct farm losses. ASFV has infected over 500 000 swine in Europe, and a significantly larger amount of swine were preventively culled.

Vaccination with baculoviral-expressed African Swine Fever Virus proteins EP402R/CD2v demonstrated an important degree of protection against homologous African Swine Fever Virus challenge (Ruiz-Gonzalvo et al., 1996, Virology, 218(1):285-289). However, immunization of pigs with recombinant p54 and p30 proteins, which are involved in steps of virus attachment and internalization, expressed in baculoviral did not protect against virulent African Swine Fever Virus challenge. Proteins encoding p30, p54, p22 and

p72 generated in baculoviral also failed to protect pigs against viral challenge, despite producing neutralizing antibodies (Neilan et al., 2004, Virology, 319(2):337-342).

DNA immunogens for p30 and p54 linked to a single variable chain of an antibody recognizing swine leukocyte antigen II, for *in vivo* immune targeting was tested. While Specific T cells against African Swine Fever Virus proteins could be induced at low levels; neither neutralizing antibodies nor protection against a virulent challenge was achieved (Argilaguet et al., 2011, Vaccine, 29: 5379–5385). A study of a DNA EC domain of HA fused to viral p30and p54 proteins was performed. Induction of both humoral and cellular immune responses (likely CD4 responses) in pigs were induced without protection against African Swine Fever Virus challenge (Argilaguet et al., 2012, PLoS One, 7(9): e40942).

An approach to improve antigen processing was undertaken. DNA constructs (ASFV p54 and p30 fused to ubiquitin) were studied. This vaccine conferred partial protection against challenge in the absence of African Swine Fever Virus-specific antibodies; protection correlated with the T cell response, and antigen-specific CD8+T cells to p54 and p30 antigens were observed (Argilaguet et al., 2012, PLoS One, 7(9): e40942; Lacasta et al., 2014, J Virol, 88(22):13322-13332).

These results of current approaches for African Swine Fever Virus vaccines add to the confusion regarding the role of antibody-mediated neutralization in African Swine Fever Virus protection (Escribano et al., 2013, Virus Res, 173(1):101-109).

Therefore, there remains a need to develop a vaccine for prophylaxis against and treatment of ASFV. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one embodiment, the invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen.

In one embodiment, the nucleic acid molecule comprises at least one nucleotide sequence encoding a peptide comprising an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the nucleic acid molecule comprises an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the nucleic acid

molecule comprises at least one nucleotide sequence encoding a peptide comprising an amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the nucleic acid molecule comprises an immunogenic fragment comprising at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30.

In one embodiment, the nucleic acid molecule is a DNA molecule or an RNA molecule.

In one embodiment, the nucleic acid molecule comprises a nucleotide sequence having at least about 90% identity over an entire length of a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29. In one embodiment, the nucleic acid molecule comprises an immunogenic fragment of a nucleotide sequence having at least about 90% identity over at least 60% of the nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29. In one embodiment, the nucleic acid molecule comprises a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29. In one embodiment, the nucleic acid molecule comprises an immunogenic fragment of a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29.

In one embodiment, the encoded peptide is operably linked to at least one regulatory sequence selected from a start codon, an IgE leader sequence and a stop codon.

In one embodiment, the nucleic acid molecule encodes at least two peptides comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30, b) an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30, c) the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30, and d) an immunogenic fragment comprising at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30.

In one embodiment, the nucleic acid molecule encodes an amino acid sequence selected from the group consisting of: a) an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence selected from the group

consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20; b) an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20; c) the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20; and d) an immunogenic fragment comprising at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20.

In one embodiment, the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of: a) a nucleotide sequence having at least about 90% identity over an entire length of a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19; b) an immunogenic fragment of a nucleotide sequence having at least about 90% identity over at least 60% of the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19; c) a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19; and d) an immunogenic fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19.

In one embodiment, the nucleic acid molecule comprises an expression vector. In one embodiment, the nucleic acid molecule comprises a viral particle.

In one embodiment, the invention related to an immunogenic composition comprising at least one nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen.

In one embodiment, the composition comprises at least one nucleotide sequence encoding a peptide comprising an amino acid sequence having at least about 90%

identity over an entire length of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the composition comprises an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the composition comprises at least one nucleotide sequence encoding a peptide comprising an amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the composition comprises an immunogenic fragment comprising at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30.

In one embodiment, the composition comprises a DNA molecule or an RNA molecule.

In one embodiment, the composition comprises a nucleotide sequence having at least about 90% identity over an entire length of a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29. In one embodiment, the composition comprises an immunogenic fragment of a nucleotide sequence having at least about 90% identity over at least 60% of the nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29. In one embodiment, the composition comprises a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29. In one embodiment, the composition comprises an immunogenic fragment of a nucleotide sequence of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 or SEQ ID NO:29.

In one embodiment, the immunogenic composition further comprises a pharmaceutically acceptable excipient. In one embodiment, the immunogenic composition further comprises an adjuvant.

In one embodiment, the invention relates to a peptide comprising an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the invention relates to a peptide comprising an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the invention relates to a peptide comprising the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one

embodiment, the invention relates to a peptide comprising an immunogenic fragment comprising at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30.

In one embodiment, the invention relates to an immunogenic composition comprising a peptide comprising an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the invention relates to an immunogenic composition comprising a peptide comprising an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the invention relates to an immunogenic composition comprising a peptide comprising the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30. In one embodiment, the invention relates to an immunogenic composition comprising a peptide comprising an immunogenic fragment comprising at least 60% of the amino acid sequence of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 or SEQ ID NO:30.

In one embodiment, the invention relates a method of inducing an immune response against an ASFV antigen in a subject in need thereof, the method comprising administering a nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen or an immunogenic composition comprising a nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen. In one embodiment, the method ofadministering includes at least one of electroporation and injection.

In one embodiment, the invention relates a method of treating or preventing an ASFV associated pathology in subject in need thereof, the method comprising administering a nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen or an immunogenic composition comprising a nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen. In one embodiment, the method ofadministering includes at least one of electroporation and injection. In one embodiment, the ASFV associated pathology is lethal hemorrhagic fever.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the design for synthetic enhanced DNA vaccine (SEV) constructs for ASFV.

Figure 2 depicts an exemplary immunization schedule used for sera staining experiments.

Figure 3 depicts exemplary images depicting control sera staining on ASFV antigen expressing cells.

Figure 4 depicts exemplary images depicting Group 1 (ASFV-p32+p54) immune sera staining.

Figure 5 depicts exemplary images depicting Group 2 (ASFV-CD2+p32+p54) immune sera staining.

Figure 6 depicts exemplary images depicting Group 3 (ASFV-Ubiquitin+CD2+p32+p54) immune sera staining.

Figure 7 depicts an exemplary immunization schedule used for serology experiments.

Figure 8 depicts exemplary experimental results demonstrating serology against matched p32 peptides.

Figure 9 depicts exemplary experimental results demonstrating serology against matched p54 peptides.

Figure 10 depicts exemplary experimental results demonstrating serology against matched CD2 peptides.

Figure 11 depicts exemplary experimental results demonstrating the cellular immune responses elicited by ASFV-DNA vaccines.

Figure 12, comprising Figure 12A and Figure 12B, depicts exemplary experimental data demonstrating the results of an intracellular cytokine staining assay (ICS)-ASFV-CD2+p32+p54. Figure 12A depicts the responses to CD4 epitopes. Figure 12B depicts the responses to CD8 epitopes.

Figure 13, comprising Figure 13A and Figure 13B, depicts exemplary experimental data demonstrating the results of an intracellular cytokine staining assay (ICS)-ASFV-ubiquitin+CD2+p32+p54. Figure 13A depicts the responses to CD4 epitopes. Figure 13B depicts the responses to CD8 epitopes.

DETAILED DESCRIPTION

The present invention relates to a composition comprising a recombinant nucleic acid sequence that encodes at least one African swine fever viral (ASFV) antigen, and functional fragments thereof. The composition can be administered to a subject in need thereof to elicit an immune response in the subject against ASFV virus.

In one embodiment, the composition comprises one or more nucleotide sequences capable of expressing one or more synthetic ASFV antigens in the subject and a pharmaceutically acceptable excipient. In one embodiment, one or more synthetic ASFV antigen is one or more of ASFV p32, p54, p12, p72 and CD2. In one embodiment, the nucleic acid molecule comprises a promoter operably linked to a coding sequence that encodes two or more synthetic ASFV antigens. In one embodiment, the nucleic acid molecule encodes two or more of synthetic ASFV p32, p54, p12, p72 and CD2 antigens. In one embodiment, the nucleic acid molecule encodes synthetic ASFV p32 and p54. In one embodiment, the nucleic acid molecule encodes each of synthetic ASFV p32, p54 and CD2. In one embodiment, the invention relates to a single nucleic acid construct for expression of each of synthetic ASFV p32, p54 and p12. In one embodiment, the nucleic acid molecule encodes each of synthetic ASFV CD2, p32, p54 and p72. In one embodiment, the nucleic acid construct encodes a synthetic ubiquitin sequence. In one embodiment, the nucleic acid molecule encodes synthetic ubiquitin and synthetic ASFV p32, p54 and CD2.

Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

The terms “comprise(s),” “include(s),” “having,” “has,” “can,” “contain(s),” and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms “a,” “and” and “the” include plural references unless the context clearly dictates otherwise. The present disclosure also contemplates other embodiments

"comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

"Adjuvant" as used herein may mean any molecule added to a nucleic acid vaccine to enhance antigenicity of the vaccine.

"Antigen" refers to proteins that have the ability to generate an immune response in a host. An antigen may be recognized and bound by an antibody. An antigen may originate from within the body or from the external environment.

"Coding sequence" or "encoding nucleic acid" as used herein may mean refers to the nucleic acid (RNA or DNA molecule) that comprise a nucleotide sequence which encodes an antigen as set forth herein. The coding sequence may further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to whom the nucleic acid is administered. The coding sequence may further include sequences that encode signal peptides.

"Complement" or "complementary" as used herein may mean a nucleic acid may mean Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

"Consensus" or "consensus sequence" as used herein may mean a synthetic nucleotide sequence, or corresponding polypeptide sequence, constructed based on analysis of an alignment of multiple sequences (e.g., multiple sequences of a particular virus antigen.)

The term "constant current" is used herein to define a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein. This current remains at a constant amperage in said tissue over the life of an electrical pulse because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (e.g., increase voltage) so current in same tissue remains constant throughout the electrical pulse (on the order of microseconds), and from pulse to pulse. In some embodiments, the feedback element comprises a controller.

"Current feedback" or "feedback" as used herein may be used interchangeably and may mean the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered

by the EP device accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback may be accomplished by the electroporation component, e.g., controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop may be instantaneous as it is an analog closed-loop feedback.

"Decentralized current" as used herein may mean the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

"Electroporation," "electro-permeabilization," or "electro-kinetic enhancement" ("EP") as used interchangeably herein may refer to the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

"Endogenous antibody" as used herein may refer to an antibody that is generated in a subject that is effective for induction of a humoral immune response.

"Feedback mechanism" as used herein may refer to a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value, preferably current, and adjusts the pulse of energy delivered to achieve the preset value. A feedback mechanism may be performed by an analog closed loop circuit.

"Fragment" may mean a percentage of a full-length polypeptide sequence or nucleotide sequence. Fragments may comprise 20% or more, 25% or more, 30% or more, 35% or more, 40% or more, 45% or more, 50% or more, 55% or more, 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, 99% or more percent of the full-length of the parental nucleotide sequence or amino acid sequence or variant thereof.

"Genetic construct" as used herein refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein, such as an antigen. The coding

sequence includes initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

"Impedance" as used herein may be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

"Immune response" as used herein may mean the activation of a host's immune system, e.g., that of a mammal, in response to the introduction of one or more nucleic acids and/or peptides. The immune response can be in the form of a cellular or humoral response, or both.

"Nucleic acid" or "oligonucleotide" or "polynucleotide" as used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may hybridize to a target sequence under stringent hybridization conditions. Thus,

a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

"Operably linked" as used herein may mean that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene may be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

A "peptide," "protein," or "polypeptide" as used herein can mean a linked sequence of amino acids and can be natural, synthetic, or a modification or combination of natural and synthetic.

"Promoter" as used herein may mean a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter may comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter may also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A promoter may be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter may regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV 40 late promoter and the CMV IE promoter.

"Signal peptide" and "leader sequence" are used interchangeably herein and refer to an amino acid sequence that can be linked at the amino terminus of a protein set forth herein. Signal peptides/leader sequences typically direct localization of a protein. Signal peptides/leader sequences used herein preferably facilitate secretion of the protein from the cell in which it is produced. Signal peptides/leader sequences are often cleaved from the remainder of the protein, often referred to as the mature protein, upon secretion from the cell. Signal peptides/leader sequences are linked at the N terminus of the protein.

"Subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal (e.g., cow, pig, camel, llama, horse, goat, rabbit, sheep, hamsters, guinea pig, cat, dog, rat, and mouse, a non-human primate (for example, a monkey, such as a cynomolgous or rhesus monkey, chimpanzee, etc.) and a human). In some embodiments, the subject may be a domestic pig.

"Substantially complementary" as used herein may mean that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

"Substantially identical" as used herein may mean that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,or 99% over a region of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100 or more nucleotides or amino acids, or with respect to nucleic acids, if the first sequence is substantially complementary to the complement of the second sequence.

The term "subtype" or "serotype" is used herein interchangeably and, in reference to a virus, means genetic variants of that virus antigen such that one subtype is recognized by an immune system apart from a different subtype.

"Treatment" or "treating," as used herein can mean protecting of a subject from a disease through means of preventing, suppressing, repressing, or completely eliminating the disease. Preventing the disease involves administering a vaccine of the present invention to a subject prior to onset of the disease. Suppressing the disease involves administering a vaccine of the present invention to a subject after induction of the disease but

before its clinical appearance. Repressing the disease involves administering a vaccine of the present invention to a subject after clinical appearance of the disease.

"Variant" used herein with respect to a nucleic acid may mean (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

"Variant" with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant may also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side

chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

A variant may be a nucleic acid sequence that is substantially identical over the full length of the full gene sequence or a fragment thereof. The nucleic acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the gene sequence or a fragment thereof. A variant may be an amino acid sequence that is substantially identical over the full length of the amino acid sequence or fragment thereof. The amino acid sequence may be 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the full length of the amino acid sequence or a fragment thereof.

"Vector" as used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

Description

The invention is based, in part on the development of an optimized synthetic sequence encoding one or more African swine fever virus (ASFV) antigen. In one embodiment, the one or more ASFV antigen encoded by the optimized synthetic sequence is capable of eliciting an immune response in a mammal. In one embodiment, the ASFV antigen encoded by the optimized synthetic sequence can comprise an epitope(s) that makes it particularly effective as an immunogen against which an immune response can be induced.

In some embodiments, the synthetic sequence is a consensus sequence derived from two or more ASFV antigens. In some embodiments, the synthetic sequence comprises a consensus sequence and/or one or more modification(s) for improved expression. Modification can include codon optimization, RNA optimization, addition of a Kozak

sequence for increased translation initiation, and/or the addition of an immunoglobulin leader sequence to increase immunogenicity. The ASFV antigen encoded by the synthetic sequence can comprise a signal peptide such as an immunoglobulin signal peptide, for example, but not limited to, an immunoglobulin E (IgE) or immunoglobulin (IgG) signal peptide. In some embodiments, the antigen encoded by the synthetic sequence can comprise a hemagglutinin (HA) tag. The antigen encoded by the synthetic sequence can be designed to elicit stronger cellular and/or humoral immune responses than a corresponding native (non-synthetic) antigen.

Provided herein are ASFV antigens that can be used to induce immunity against ASFV in subjects with or at risk of ASFV infection. In one embodiment, the present invention provides an immunogenic composition comprising one or more nucleic acid molecules that are capable of generating in a mammal an immune response against an ASFV antigen. The present invention also provides isolated nucleic acid molecules that are capable of generating in a mammal an immune response against an ASFV antigen. In one embodiment, the nucleic acid molecule comprises an optimized nucleotide sequence encoding a synthetic ASFV antigen.

Optimized Synthetic ASFV antigens

In one embodiment, the present invention provides an immunogenic composition comprising one or more nucleic acid molecules that are capable of generating in a mammal an immune response against an ASFV antigen. The present invention also provides isolated nucleic acid molecules that are capable of generating in a mammal an immune response against an ASFV antigen. In one embodiment, the nucleic acid molecule comprises an optimized nucleotide sequence encoding at least 1, 2, 3 or more than 3 synthetic ASFV antigens. In one embodiment, one or more synthetic antigens are synthetic ASFV p32, p54, p12, p72 or CD2 antigens, fragments thereof, variants thereof, or any combination thereof.

Synthetic amino acid sequences for ASFV antigens include SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, and SEQ ID NO:30 and variants thereof and fragments of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, and SEQ ID NO:30, and variants thereof. An exemplary amino acid sequence of a synthetic ASFV p32 antigen is provided as SEQ ID NO:26. An exemplary amino acid sequence of a synthetic ASFV p54 antigen is provided as SEQ ID NO:28. An exemplary amino acid sequence of a

synthetic ASFV p12 antigen is provided as SEQ ID NO:24. An exemplary amino acid sequence of a synthetic ASFV CD2 antigen is provided as SEQ ID NO:22. An exemplary amino acid sequence of a synthetic ASFV CD2 antigen is provided as SEQ ID NO:30.

In one embodiment, the invention provides compositions comprising a nucleic acid molecule comprising a nucleotide sequence that encodes a synthetic ASFV antigen. In one embodiment, a nucleotide sequence which encodes a synthetic ASFV p32 antigen is provided as SEQ ID NO:25, which encodes SEQ ID NO:26. In one embodiment, a nucleotide sequence which encodes a synthetic ASFV p54 antigen is provided as SEQ ID NO:27, which encodes SEQ ID NO:28. In one embodiment, a nucleotide sequence which encodes a synthetic ASFV p12 antigen is provided as SEQ ID NO:23, which encodes SEQ ID NO:24. In one embodiment, a nucleotide sequence which encodes a synthetic ASFV CD2 antigen is provided as SEQ ID NO:21, which encodes SEQ ID NO:22. In one embodiment, a nucleotide sequence which encodes a synthetic ASFV p72 antigen is provided as SEQ ID NO:29, which encodes SEQ ID NO:30.

In various embodiments, the invention provides compositions comprising a combination of at least two synthetic ASFV antigen, or one or more nucleic acid molecules encoding the same. The compositions may comprise a plurality of copies of a single nucleic acid molecule such a single plasmid, or a plurality of copies of two or more different nucleic acid molecules such as two or more different plasmids.

Compositions that comprise one or more nucleotide sequence that encode multiple synthetic ASFV antigens may be on a single plasmid. In some embodiments, the sequence that encodes a first ASFV antigen and the sequence that encodes at least one additional ASFV antigen may be linked by a fusion peptide sequence, for example a furin cleavage sequence. In one embodiment, a composition comprises a single plasmid that encodes synthetic ASFV p32 and p54 antigens under a single promoter. An exemplary nucleotide sequence of a single construct encoding synthetic p32 and p54 antigens encodes SEQ ID NO:2, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding synthetic p32 and p54 antigens is provided as SEQ ID NO:1, a fragment thereof or a variant thereof. In one embodiment, a composition comprises a single plasmid that encodes synthetic ASFV p32 and p54 antigens under a single promoter operably linked to an IgE leader sequence. An exemplary nucleotide sequence of a single construct encoding synthetic p32 and p54 antigens operably linked to an IgE leader sequence encodes SEQ ID NO:4, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of

a single construct encoding synthetic p32 and p54 antigens operably linked to an sequence encoding an IgE leader sequence is provided as SEQ ID NO:3, a fragment thereof or a variant thereof.

In one embodiment, a composition comprises a single plasmid that encodes ASFV ubiquitin+CD2+p32+ p54 antigens under a single promoter. An exemplary nucleotide sequence of a single construct encoding ASFV ubiquitin+CD2+p32+ p54 antigens encodes SEQ ID NO:6, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV ubiquitin+CD2+p32+ p54 antigens is provided as SEQ ID NO:5, a fragment thereof or a variant thereof. In one embodiment, a composition comprises a single plasmid that encodes ASFV ubiquitin+CD2+p32+ p54 antigens under a single promoter operably linked to an IgE leader sequence. An exemplary nucleotide sequence of a single construct encoding ASFV ubiquitin+CD2+p32+ p54 antigens operably linked to an IgE leader sequence encodes SEQ ID NO:8, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV ubiquitin+CD2+p32+ p54 antigens operably linked to an sequence encoding an IgE leader sequence is provided as SEQ ID NO:7, a fragment thereof or a variant thereof.

In one embodiment, a composition comprises a single plasmid that encodes ASFV CD2+p32+ p54 antigens under a single promoter. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+p32+ p54 antigens encodes SEQ ID NO:10, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+p32+ p54 antigens is provided as SEQ ID NO:9, a fragment thereof or a variant thereof. In one embodiment, a composition comprises a single plasmid that encodes ASFV CD2+p32+ p54 antigens under a single promoter operably linked to an IgE leader sequence. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+p32+ p54 antigens operably linked to an IgE leader sequence encodes SEQ ID NO:12, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+p32+ p54 antigens operably linked to an sequence encoding an IgE leader sequence is provided as SEQ ID NO:11, a fragment thereof or a variant thereof.

In one embodiment, a composition comprises a single plasmid that encodes ASFV p32+ p12+p54 antigens under a single promoter. An exemplary nucleotide sequence of a single construct encoding ASFV p32+ p12+p54 antigens encodes SEQ ID NO:14, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV p32+ p12+p54 antigens is provided as SEQ ID NO:13, a fragment thereof or

a variant thereof. In one embodiment, a composition comprises a single plasmid that encodes ASFV p32+ p12+p54 antigens under a single promoter operably linked to an IgE leader sequence. An exemplary nucleotide sequence of a single construct encoding ASFV p32+ p12+p54 antigens operably linked to an IgE leader sequence encodes SEQ ID NO:16, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV p32+ p12+p54 antigens operably linked to a sequence encoding an IgE leader sequence is provided as SEQ ID NO:15, a fragment thereof or a variant thereof.

In one embodiment, a composition comprises a single plasmid that encodes ASFV CD2+ p32+ p54 +p72 antigens under a single promoter. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+ p32+ p54 +p72 antigens encodes SEQ ID NO:18, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+ p32+ p54 +p72 antigens is provided as SEQ ID NO:17, a fragment thereof or a variant thereof. In one embodiment, a composition comprises a single plasmid that encodes ASFV CD2+ p32+ p54 +p72 antigens under a single promoter operably linked to an IgE leader sequence. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+ p32+ p54 +p72 antigens operably linked to an IgE leader sequence encodes SEQ ID NO:20, a fragment thereof or a variant thereof. An exemplary nucleotide sequence of a single construct encoding ASFV CD2+ p32+ p54 +p72 antigens operably linked to a sequence encoding an IgE leader sequence is provided as SEQ ID NO:19, a fragment thereof or a variant thereof.

In one embodiment, the invention provides compositions comprising a nucleic acid molecule comprising a nucleotide sequence that encodes a ubiquitin targeting sequence, or a variant or fragment thereof. In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding SEQ ID NO:32, or a variant or fragment thereof. In one embodiment, the nucleic acid molecule comprises a nucleotide sequence as set forth in SEQ ID NO:31, or a variant or fragment thereof.

In one embodiment, an optimized synthetic encoded ASFV antigen is operably linked to one or more regulatory elements. In one embodiment, a regulatory element is a leader sequence. In one embodiment, the leader sequence is an IgE leader sequence. In one embodiment, the IgE leader sequence has an amino acid sequence as set forth in SEQ ID NO:33. Therefore in one embodiment, the invention relates to an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 or SEQ ID

NO:32 operably linked to an amino acid sequence as set forth in SEQ ID NO:33. In one embodiment, the invention relates to a nucleotide sequence encoding an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:18, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 or SEQ ID NO:32 operably linked to a nucleotide sequence encoding an amino acid sequence as set forth in SEQ ID NO:33.

In one embodiment, a regulatory element is a start codon. Therefore, in one embodiment, the invention relates to a nucleotide sequence as set forth in SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:31 or a fragment or variant thereof, operably linked to a nucleotide sequence comprising a start codon at the 5' terminus. In one embodiment, the invention relates to an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32 or a fragment or variant thereof, operably linked to an amino acid encoded by a start codon (e.g., a Methionine) at the N-terminus.

In one embodiment, a regulatory element is at least one stop codon. Therefore, in one embodiment, the invention relates to a nucleotide sequence as set forth in SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:31 or a fragment or variant thereof, operably linked to a nucleotide sequence comprising at least one stop codon at the 3' terminus. In one embodiment, the nucleotide sequence is operably linked to two stop codons to increase the efficiency of translational termination.

In one embodiment, nucleic acid molecule can encode at least one peptide having the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32. In one embodiment, the nucleic acid molecule comprises at least one nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31. In some embodiments, the sequence can be the nucleotide

sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31. In other embodiments, sequence can be the nucleotide sequence that encodes the amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32.

In some embodiments, the nucleic acid molecule comprises an RNA sequence that is a transcript from a DNA sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the nucleotide sequence set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31. In some embodiments, the nucleic acid molecule comprises an RNA sequence that encodes an amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity over an entire length of the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32.

The synthetic-ASFV antigen can be a peptide having the amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32. In some embodiments, the antigen can have an amino acid sequence having at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity over an entire length of the amino acid sequence set

forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32.

Immunogenic fragments of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32 can be provided. Immunogenic fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of the full length of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32. In some embodiments, immunogenic fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, immunogenic fragments are free of a leader sequence.

Immunogenic fragments of proteins with amino acid sequences homologous to immunogenic fragments of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32, can be provided. Such immunogenic fragments can comprise at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of proteins that are 95% homologous to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32. Some embodiments relate to immunogenic fragments that have 96% homology to the immunogenic fragments of synthetic protein sequences herein. Some embodiments relate to immunogenic fragments that have 97% homology to the immunogenic fragments of synthetic protein sequences herein. Some embodiments relate to immunogenic fragments that have 98% homology to the immunogenic fragments of synthetic protein sequences herein. Some embodiments relate to immunogenic fragments that have 99% homology to the immunogenic fragments of synthetic protein sequences herein. In some embodiments, immunogenic

fragments include a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, immunogenic fragments are free of a leader sequence.

Some embodiments relate to immunogenic fragments of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31 comprising at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of the full length of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31. Immunogenic fragments can be at least 96%, at least 97% at least 98% or at least 99% homologous to fragments of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31. In some embodiments, immunogenic fragments include sequences that encode a leader sequence, such as for example an immunoglobulin leader, such as the IgE leader. In some embodiments, fragments are free of coding sequences that encode a leader sequence.

In one embodiment, the nucleic acid molecule comprises a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31.

In one embodiment, the nucleic acid molecule comprises an RNA sequence encoding a synthetic ASFV antigen sequence described herein. For example, nucleic acids may comprise an RNA sequence encoding one or more of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32, a variant thereof, a fragment thereof or any combination thereof.

Nucleic Acid Constructs

When taken up by a cell, the DNA plasmids can remain in the cell as separate genetic material. Alternatively, RNA may be administered to the cell. It is also contemplated to provide a genetic construct as a linear mini chromosome including a centromere, telomeres and an origin of replication. Genetic constructs include regulatory elements necessary for gene expression of a nucleic acid molecule. The elements include: a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers are often required for gene expression of the sequence that encodes the target protein or the immunomodulating protein. It is necessary that these elements be operable linked to the sequence that encodes the desired proteins and that the regulatory elements are operably in the individual to whom they are administered. Such genetic constructs may be therefore be recombinant nucleic acid molecules.

The recombinant nucleic acid molecule can include one or more recombinant nucleotide sequence constructs. The recombinant nucleotide sequence construct can include one or more components, which are described in more detail below.

The recombinant nucleotide sequence construct can include a heterologous nucleotide sequence that encodes a viral antigen, a fragment thereof, a variant thereof, or a combination thereof. The recombinant nucleotide sequence construct can also include a heterologous nucleotide sequence that encodes a protease or peptidase cleavage site. The recombinant nucleotide sequence construct can also include a heterologous nucleotide sequence that encodes an internal ribosome entry site (IRES). An IRES may be either a viral IRES or an eukaryotic IRES. The recombinant nucleotide sequence can include one or more leader sequences, in which each leader sequence encodes a signal peptide. The recombinant nucleotide sequence can include one or more promoters, one or more introns, one or more transcription termination regions, one or more initiation codons, one or more termination or stop codons, and/or one or more polyadenylation signals. The recombinant nucleotide sequence construct can also include one or more linker or tag sequences. The tag sequence can encode a hemagglutinin (HA) tag.

a) Protease Cleavage Site

The recombinant nucleotide sequence construct can include heterologous nucleotide sequence encoding a protease cleavage site. The protease cleavage site can be recognized by a protease or peptidase. The protease can be an endopeptidase or endoprotease, for example, but not limited to, furin, elastase, HtrA, calpain, trypsin, chymotrypsin, trypsin, and pepsin. The protease can be furin. In other embodiments, the protease can be a serine

protease, a threonine protease, cysteine protease, aspartate protease, metalloprotease, glutamic acid protease, or any protease that cleaves an internal peptide bond (i.e., does not cleave the N-terminal or C-terminal peptide bond).

The protease cleavage site can include one or more amino acid sequences that promote or increase the efficiency of cleavage. The one or more amino acid sequences can promote or increase the efficiency of forming or generating discrete polypeptides. The one or more amino acids sequences can include a furin cleavage site.

b) Linker Sequence

The recombinant nucleotide sequence construct can include one or more linker sequences. The linker sequence can spatially separate or link the one or more components described herein. In other embodiments, the linker sequence can encode an amino acid sequence that spatially separates or links two or more polypeptides.

c) Promoter

The recombinant nucleotide sequence construct can include one or more promoters. The one or more promoters may be any promoter that is capable of driving gene expression and regulating gene expression. Such a promoter is a cis-acting sequence element required for transcription via a DNA dependent RNA polymerase. Selection of the promoter used to direct gene expression depends on the particular application. The promoter may be positioned about the same distance from the transcription start in the recombinant nucleotide sequence construct as it is from the transcription start site in its natural setting. However, variation in this distance may be accommodated without loss of promoter function.

The promoter may be operably linked to the heterologous nucleotide sequence encoding one or more viral antigen. The promoter may be a promoter shown effective for expression in eukaryotic cells. The promoter operably linked to the coding sequence may be a CMV promoter, a promoter from simian virus 40 (SV40), such as SV40 early promoter and SV40 later promoter, a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter may also be a promoter from a human gene such as human actin, human myosin, human hemoglobin, human muscle creatine, human polyhedrin, or human metalothionein.

The promoter can be a constitutive promoter or an inducible promoter, which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, the promoter can also be specific to a particular tissue or organ or stage of development. The promoter may also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no. US20040175727, the contents of which are incorporated herein in its entirety.

The promoter can be associated with an enhancer. The enhancer can be located upstream of the coding sequence. The enhancer may be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, FMDV, RSV or EBV. Polynucleotide function enhances are described in U.S. Patent Nos. 5,593,972, 5,962,428, and W094/016737, the contents of each are fully incorporated by reference.

d) Transcription Termination Region

The recombinant nucleotide sequence construct can include one or more transcription termination regions. The transcription termination region can be downstream of the coding sequence to provide for efficient termination. The transcription termination region can be obtained from the same gene as the promoter described above or can be obtained from one or more different genes.

e) Initiation Codon

The recombinant nucleotide sequence construct can include one or more initiation codons. The initiation codon can be located upstream of the coding sequence. The initiation codon can be in frame with the coding sequence. The initiation codon can be associated with one or more signals required for efficient translation initiation, for example, but not limited to, a ribosome binding site.

f) Termination Codon

The recombinant nucleotide sequence construct can include one or more termination or stop codons. The termination codon can be downstream of the coding sequence. The termination codon can be in frame with the coding sequence. The termination codon can be associated with one or more signals required for efficient translation termination. Initiation codons and stop codon are generally considered to be part of a nucleotide sequence that encodes the desired protein. However, it is necessary that these

elements are functional in the mammals to whom the nucleic acid construct is administered. The initiation and termination codons must be in frame with the coding sequence.

g) Polyadenylation Signal

The recombinant nucleotide sequence construct can include one or more polyadenylation signals. The polyadenylation signal can include one or more signals required for efficient polyadenylation of the transcript. The polyadenylation signal can be positioned downstream of the coding sequence. The polyadenylation signal may be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal may be a polyadenylation signal from a pCEP4 plasmid (Invitrogen, San Diego, CA). Promoters and polyadenylation signals used must be functional within the cells of the individual.

h) Leader Sequence

The recombinant nucleotide sequence construct can include one or more leader sequences. The leader sequence can encode a signal peptide. The signal peptide can be an immunoglobulin (Ig) signal peptide, for example, but not limited to, an IgG signal peptide and a IgE signal peptide.

In addition to regulatory elements required for DNA expression, as described above, other elements may also be included in the recombinant nucleic acid molecule. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human actin, human myosin, human hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

Genetic constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. Plasmids pMV101, pCEP4 and pREP4 contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region which produces high copy episomal replication without integration.

In order to maximize protein production, regulatory sequences may be selected which are well suited for gene expression in the cells the construct is administered into. Moreover, codons that encode said protein may be selected which are most efficiently transcribed in the host cell. One having ordinary skill in the art can produce DNA constructs that are functional in the cells.

In some embodiments, nucleic acid constructs may be provided in which the coding sequences for the proteins described herein are linked to IgE leader peptide, or such IgE leader is removed. In some embodiments, proteins described herein are linked to IgE signal peptide, or such IgE leader is removed.

In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, produce and isolate proteins of the invention using well known techniques. In some embodiments for which protein is used, for example, one having ordinary skill in the art can, using well known techniques, inserts DNA molecules that encode a protein of the invention into a commercially available expression vector for use in well-known expression systems. For example, the commercially available plasmid pSE420 (Invitrogen, San Diego, Calif.) may be used for production of protein in Escherichia coli (E.coli). The commercially available plasmid pYES2 (Invitrogen, San Diego, Calif.) may, for example, be used for production in Saccharomyces cerevisiae strains of yeast. The commercially available MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.) may, for example, be used for production in insect cells. The commercially available plasmid pcDNA3.I or pcDNA3 (Invitrogen, San Diego, Calif.) may, for example, be used for production in mammalian cells such as Chinese hamster ovary (CHO) cells. One having ordinary skill in the art can use these commercial expression vectors and systems or others to produce protein by routine techniques and readily available starting materials. (See e.g., Sambrook et al., Molecular Cloning a Laboratory Manual, Second Ed. Cold Spring Harbor Press (1989)). Thus, the desired proteins can be prepared in both prokaryotic and eukaryotic systems, resulting in a spectrum of processed forms of the protein.

Vector

The recombinant nucleotide sequence construct described above can be placed in one or more vectors. The one or more vectors can contain an origin of replication. The one or more vectors can be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. The one or more vectors can be either a self-replication extra chromosomal vector, or a vector which integrates into a host genome.

The one or more vectors can be a heterologous expression construct, which is generally a plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the heavy chain polypeptide and/or light chain polypeptide that are encoded by the recombinant nucleotide sequence construct is produced

by the cellular-transcription and translation machinery ribosomal complexes. The one or more vectors can express large amounts of stable messenger RNA, and therefore proteins.

i) Expression Vector

The one or more vectors can be a circular plasmid or a linear nucleic acid. The circular plasmid and linear nucleic acid are capable of directing expression of a particular nucleotide sequence in an appropriate subject cell. The one or more vectors comprising the recombinant nucleotide sequence construct may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components.

j) Plasmid

The one or more vectors can be a plasmid. The plasmid may be useful for transfecting cells with the recombinant nucleotide sequence construct. The plasmid may be useful for introducing the recombinant nucleotide sequence construct into the subject. The plasmid may also comprise a regulatory sequence, which may be well suited for gene expression in a cell into which the plasmid is administered.

k) RNA

In one embodiment, the nucleic acid is an RNA molecule. In one embodiment, the RNA molecule is transcribed from a DNA sequence described herein. For example, in some embodiments, the RNA molecule is encoded by a DNA sequence at least 90% homologous to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, or SEQ ID NO:31. In another embodiment, the nucleotide sequence comprises an RNA sequence transcribed by a DNA sequence encoding a polypeptide sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, or SEQ ID NO:32, or a variant thereof or a fragment thereof. Accordingly, in one embodiment, the invention provides an RNA molecule encoding one or more synthetic ASFV antigen. The RNA may be plus-stranded. Accordingly, in some embodiments, the RNA molecule can be translated by cells without needing any intervening replication steps such as reverse transcription. An RNA molecule useful with the invention may have a 5′ cap (e.g. a 7-methylguanosine). This cap can enhance in vivo translation of the RNA. The 5′ nucleotide of a RNA molecule useful with the invention may have a 5′ triphosphate group. In a capped RNA this may be linked to a 7-methylguanosine via a 5′-to-5′ bridge. An RNA

molecule may have a 3′ poly-A tail. It may also include a poly-A polymerase recognition sequence (e.g. AAUAAA) near its 3′ end. A RNA molecule useful with the invention may be single-stranded. A RNA molecule useful with the invention may comprise synthetic RNA. In some embodiments, the RNA molecule is a naked RNA molecule. In one embodiment, the RNA molecule is comprised within a vector.

In one embodiment, the RNA has 5' and 3' UTRs. In one embodiment, the 5' UTR is between zero and 3000 nucleotides in length. The length of 5' and 3' UTR sequences to be added to the coding region can be altered by different methods, including, but not limited to, designing primers for PCR that anneal to different regions of the UTRs. Using this approach, one of ordinary skill in the art can modify the 5' and 3' UTR lengths required to achieve optimal translation efficiency following transfection of the transcribed RNA.

The 5' and 3' UTRs can be the naturally occurring, endogenous 5' and 3' UTRs for the gene of interest. Alternatively, UTR sequences that are not endogenous to the gene of interest can be added by incorporating the UTR sequences into the forward and reverse primers or by any other modifications of the template. The use of UTR sequences that are not endogenous to the gene of interest can be useful for modifying the stability and/or translation efficiency of the RNA. For example, it is known that AU-rich elements in 3' UTR sequences can decrease the stability of RNA. Therefore, 3' UTRs can be selected or designed to increase the stability of the transcribed RNA based on properties of UTRs that are well known in the art.

In one embodiment, the 5' UTR can contain the Kozak sequence of the endogenous gene. Alternatively, when a 5' UTR that is not endogenous to the gene of interest is being added by PCR as described above, a synthetic Kozak sequence can be redesigned by adding the 5' UTR sequence. Kozak sequences can increase the efficiency of translation of some RNA transcripts, but does not appear to be required for all RNAs to enable efficient translation. The requirement for Kozak sequences for many RNAs is known in the art. In other embodiments, the 5' UTR can be derived from an RNA virus whose RNA genome is stable in cells. In other embodiments, various nucleotide analogues can be used in the 3' or 5' UTR to impede exonuclease degradation of the RNA.

In one embodiment, the RNA has both a cap on the 5' end and a 3' poly(A) tail which determine ribosome binding, initiation of translation and stability of RNA in the cell.

In one embodiment, the RNA is a nucleoside-modified RNA. Nucleoside-modified RNA have particular advantages over non-modified RNA, including for example, increased stability, low or absent innate immunogenicity, and enhanced translation.

l) Circular and Linear Vector

The one or more vectors may be circular plasmid, which may transform a target cell by integration into the cellular genome or exist extra chromosomally (e.g., autonomous replicating plasmid with an origin of replication). The vector can be pVAX1, pMV101, pcDNA3.0, or provax, or any other expression vector capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleotide sequence construct.

Also provided herein is a linear nucleic acid, or linear expression cassette ("LEC"), that is capable of being efficiently delivered to a subject via electroporation and expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleotide sequence construct. The LEC may be any linear DNA devoid of any phosphate backbone. The LEC may not contain any antibiotic resistance genes and/or a phosphate backbone. The LEC may not contain other nucleotide sequences unrelated to the desired gene expression.

The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleotide sequence construct. The plasmid can be pNP (Puerto Rico/34) or pM2 (New Caledonia/99). The plasmid may be WLV009, pVAX, pMV101, pcDNA3.0, or provax, or any other expression vector capable of expressing the heavy chain polypeptide and/or light chain polypeptide encoded by the recombinant nucleotide sequence construct.

The LEC can be pcrM2. The LEC can be pcrNP. pcrNP and pcrMR can be derived from pNP (Puerto Rico/34) and pM2 (New Caledonia/99), respectively.

m) Viral Vectors

In one embodiment, viral vectors are provided herein which are capable of delivering a nucleic acid of the invention to a cell. The expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001), and in Ausubel et al. (1997), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and

lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., swine cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

n) Method of Preparing the Vector

Provided herein is a method for preparing the one or more vectors in which the recombinant nucleotide sequence construct has been placed. After the final subcloning step, the vector can be used to inoculate a cell culture in a large-scale fermentation tank, using known methods in the art.

In other embodiments, after the final subcloning step, the vector can be used with one or more electroporation (EP) devices. The EP devices are described below in more detail.

The one or more vectors can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using a plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on May 23, 2007. In some examples, the DNA plasmids described herein can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No. 60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

2. Vaccines and Immunogenic Compositions

Immunogenic compositions, such as vaccines, are provided comprising an optimized synthetic sequence, an optimized synthetic-encoded antigen, a fragment thereof, a variant thereof, or a combination thereof. The immunogenic composition can significantly induce an immune response of a subject administered with the immunogenic composition against the ASFV antigen. The vaccine may comprise a plurality of the nucleic acid

molecules, or combinations thereof. The vaccine may be provided to induce a therapeutic or prophylactic immune response.

The immunogenic composition can be a DNA vaccine, an RNA vaccine, a peptide vaccine, or a combination vaccine. The vaccine can include an optimized synthetic nucleotide sequence encoding an antigen. The nucleotide sequence can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The nucleotide sequence can also include additional sequences that encode linker, leader, or tag sequences that are linked to the antigen by a peptide bond. The peptide vaccine can include an antigen, a variant thereof, a fragment thereof, or a combination thereof. The combination DNA and peptide vaccine can include the above described optimized synthetic nucleotide sequence and the encoded antigen.

The vaccine can be a DNA vaccine. DNA vaccines are disclosed in US Patent Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, and 5,676,594, which are incorporated herein fully by reference. The DNA vaccine can further comprise elements or reagents that inhibit it from integrating into the chromosome.

The vaccine can be an RNA of the one or more ASFV antigens. The RNA vaccine can be introduced into the cell.

The vaccine can be an attenuated live vaccine, a vaccine using recombinant vectors to deliver antigen, subunit vaccines, and glycoprotein vaccines, for example, but not limited, the vaccines described in U.S. Patent Nos.: 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,3 64; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439; 5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

The vaccine of the present invention can have features required of effective vaccines such as being safe so that the vaccine itself does not cause illness or death; being protective against illness; inducing protective T cell responses; and providing ease of administration, few side effects, biological stability, and low cost per dose.

Provided herein is an immunogenic composition capable of generating in a mammal an immune response against ASFV. The immunogenic composition may comprise each plasmid as discussed above. The immunogenic composition may comprise a plurality of

the plasmids, or combinations thereof. The immunogenic composition may be provided to induce a therapeutic or prophylactic immune response.

Immunogenic compositions may be used to deliver nucleic acid molecules that encode one or more synthetic ASFV antigen. Immunogenic compositions are preferably compositions comprising plasmids.

Another aspect of the present invention provides immunogenic compositions that are capable of generating in a mammal an immune response against one or more ASFV viruses. The immunogenic compositions are comprised of one or more nucleic acid molecules capable of expressing at least one synthetic viral antigen in the mammal.

In one embodiment, the immunogenic composition comprises a nucleotide sequence that encodes at least one synthetic ASFV antigen. The synthetic viral antigens may be synthetic p32, p54, p12, p72, CD2, or a fusion of one or more of aforementioned antigens.

Each antigen can be associated with viral infection. In one embodiment, each antigen can be associated with an ASFV virus infection.

The antigen can be a nucleic acid sequence, an amino acid sequence, a polysaccharide or a combination thereof. The nucleic acid sequence can be DNA, RNA, cDNA, a variant thereof, a fragment thereof, or a combination thereof. The amino acid sequence can be a protein, a peptide, a variant thereof, a fragment thereof, or a combination thereof. The polysaccharide can be a nucleic acid encoded polysaccharide.

In some embodiments, the immunogenic composition comprises a plurality of unique nucleic acid molecules, wherein the plurality of unique nucleic acid molecules encodes each of a synthetic p32, and synthetic p54 immunogen. In some embodiments, the immunogenic composition comprises a plurality of unique nucleic acid molecules, wherein the plurality of unique nucleic acid molecules encodes each of a synthetic CD2 immunogen, synthetic p32, and synthetic p54 immunogen. In some embodiments, the immunogenic composition comprises a plurality of unique nucleic acid molecules, wherein the plurality of unique nucleic acid molecules encodes each of a synthetic p12 immunogen, synthetic p32, and synthetic p54 immunogen. In some embodiments, the immunogenic composition comprises a plurality of unique nucleic acid molecules, wherein the plurality of unique nucleic acid molecules encodes each of a synthetic CD2 immunogen, synthetic p32 immunogen, synthetic p54 immunogen and synthetic p72 immunogen.

Exemplary sequences that can be included in the immunogenic composition of the invention may be selected from:

SEQ ID NO:	Type	Description
1	Nucleotide	ASFV p32 + p54
2	Amino Acid	ASFV p32 + p54
3	Nucleotide	ASFV p32 + p54 operably linked to IgE
4	Amino Acid	ASFV p32 + p54 operably linked to IgE
5	Nucleotide	ASFV ubiquitin+CD2+p32+ p54
6	Amino Acid	ASFV ubiquitin+CD2+p32+ p54
7	Nucleotide	ASFV ubiquitin+CD2+p32+ p54 operably linked to IgE
8	Amino Acid	ASFV ubiquitin+CD2+p32+ p54 operably linked to IgE
9	Nucleotide	ASFV CD2+p32+p54
10	Amino Acid	ASFV CD2+p32+p54
11	Nucleotide	ASFV CD2+p32+p54 operably linked to IgE
12	Amino Acid	ASFV CD2+p32+p54 operably linked to IgE
13	Nucleotide	ASFV p32+p12+p54
14	Amino Acid	ASFV p32+p12+p54
15	Nucleotide	ASFV p32+p12+p54 operably linked to IgE
16	Amino Acid	ASFV p32+p12+p54 operably linked to IgE
17	Nucleotide	ASFV CD2+p32+p54+p72
18	Amino Acid	ASFV CD2+p32+p54+p72
19	Nucleotide	ASFV CD2+p32+p54+p72 operably linked to IgE
20	Amino Acid	ASFV CD2+p32+p54+p72 operably linked to IgE
21	Nucleotide	Synthetic ASFV CD2 antigen
22	Amino Acid	Synthetic ASFV CD2 antigen
23	Nucleotide	Synthetic ASFV p12 antigen
24	Amino Acid	Synthetic ASFV p12 antigen
25	Nucleotide	Synthetic ASFV p32 antigen
26	Amino Acid	Synthetic ASFV p32 antigen
27	Nucleotide	Synthetic ASFV p54 antigen
28	Amino Acid	Synthetic ASFV p54 antigen
29	Nucleotide	Synthetic ASFV p72 antigen
30	Amino Acid	Synthetic ASFV p72 antigen
31	Nucleotide	Synthetic ubiquitin

32	Amino Acid	Synthetic ubiquitin
33	Nucleotide	IgE leader sequence

In one embodiment, the nucleic acid molecule comprises an optimized nucleic acid sequence. The optimized sequence can comprise a synthetic sequence and/or modification(s) for improved expression. Modification can include codon optimization, RNA optimization, addition of a Kozak sequence for increased translation initiation, and/or the addition of an immunoglobulin leader sequence to increase immunogenicity. The ASFV antigen encoded by the optimized sequence can comprise a signal peptide such as an immunoglobulin signal peptide, for example, but not limited to, an immunoglobulin E (IgE) or immunoglobulin (IgG) signal peptide. In some embodiments, the antigen encoded by the optimized synthetic sequence can comprise a hemagglutinin (HA) tag. The ASFV antigen encoded by the optimized sequence can be designed to elicit stronger cellular and/or humoral immune responses than a corresponding native antigen.

The immunogenic composition can induce an immune response in the subject administered the composition. The induced immune response can be specific for at least one ASFV antigen. The induced immune response can be reactive with at least one ASFV antigen related to an administered optimized synthetic-encoded antigen. In various embodiments, related antigens include antigens having amino acid sequences having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homology to the amino acid sequence of the optimized synthetic-encoded antigen. In various embodiments, related antigens include antigens encoded by nucleotide sequences having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% homology to the optimized synthetic nucleotide sequences disclosed herein.

The immunogenic composition can induce a humoral immune response in the subject administered the immunogenic composition. The induced humoral immune response can be specific for at least one ASFV antigen. The induced humoral immune response can be reactive with at least one ASFV antigen related to an administered optimized synthetic-encoded antigen. The humoral immune response can be induced in the subject administered the immunogenic composition by about 1.5-fold to about 16-fold, about 2-fold to about 12-fold, or about 3-fold to about 10-fold. The humoral immune response can be induced in the subject administered the immunogenic composition by at least about 1.5-fold, at least about 2.0-fold, at least about 2.5-fold, at least about 3.0-fold, at least about 3.5-fold, at least about

4.0-fold, at least about 4.5-fold, at least about 5.0-fold, at least about 5.5-fold, at least about 6.0-fold, at least about 6.5-fold, at least about 7.0-fold, at least about 7.5-fold, at least about 8.0-fold, at least about 8.5-fold, at least about 9.0-fold, at least about 9.5-fold, at least about 10.0-fold, at least about 10.5-fold, at least about 11.0-fold, at least about 11.5-fold, at least about 12.0-fold, at least about 12.5-fold, at least about 13.0-fold, at least about 13.5-fold, at least about 14.0-fold, at least about 14.5-fold, at least about 15.0-fold, at least about 15.5-fold, or at least about 16.0- fold as compared to a subject not administered the immunogenic composition of the invention.

The humoral immune response induced by the immunogenic composition can include an increased level of IgG antibodies associated with the subject administered the immunogenic composition as compared to a subject not administered the immunogenic composition. These IgG antibodies can be specific for at least one ASFV antigen genetically related to an administered optimized synthetic-encoded antigen. These IgG antibodies can be reactive with at least one ASFV antigen genetically related to an administered optimized synthetic-encoded antigen. The level of IgG antibody associated with the subject administered the immunogenic composition can be increased by about 1.5-fold to about 16-fold, about 2-fold to about 12-fold, or about 3-fold to about 10-fold as compared to the subject not administered the immunogenic composition. The level of IgG antibody associated with the subject administered the immunogenic composition can be increased by at least about 1.5-fold, at least about 2.0-fold, at least about 2.5-fold, at least about 3.0-fold, at least about 3.5-fold, at least about 4.0-fold, at least about 4.5-fold, at least about 5.0-fold, at least about 5.5-fold, at least about 6.0-fold, at least about 6.5-fold, at least about 7.0-fold, at least about 7.5-fold, at least about 8.0-fold, at least about 8.5-fold, at least about 9.0-fold, at least about 9.5-fold, at least about 10.0-fold, at least about 10.5-fold, at least about 11.0-fold, at least about 11.5-fold, at least about 12.0-fold, at least about 12.5-fold, at least about 13.0-fold, at least about 13.5-fold, at least about 14.0-fold, at least about 14.5-fold, at least about 15.0-fold, at least about 15.5-fold, or at least about 16.0-fold as compared to a subject not administered the immunogenic composition.

The immunogenic composition can induce a cellular immune response in the subject administered the immunogenic composition. The induced cellular immune response can be specific for at least one ASFV antigen genetically related to an administered optimized synthetic-encoded antigen. The induced cellular immune response can be reactive at least one ASFV antigen genetically related to an administered optimized synthetic-encoded antigen.

The induced cellular immune response can include eliciting a CD8+ T cell response. The elicited CD8+ T cell response can be reactive with at least one ASFV antigen genetically related to an administered optimized synthetic-encoded antigen. The elicited CD8+ T cell response can be polyfunctional. The induced cellular immune response can include eliciting a CD8+ T cell response, in which the CD8+ T cells produce interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin-2 (IL-2), or a combination of IFN-γ and TNF-α.

The induced cellular immune response can include an increased CD8+ T cell response associated with the subject administered the immunogenic composition as compared to the subject not administered the immunogenic composition. The CD8+ T cell response associated with the subject administered the immunogenic composition can be increased by about 2-fold to about 30-fold, about 3-fold to about 25-fold, or about 4-fold to about 20-fold as compared to the subject not administered the immunogenic composition. The CD8+ T cell response associated with the subject administered the immunogenic composition can be increased by at least about 1.5-fold, at least about 2.0-fold, at least about 3.0-fold, at least about 4.0-fold, at least about 5.0-fold, at least about 6.0-fold, at least about 6.5-fold, at least about 7.0-fold, at least about 7.5-fold, at least about 8.0-fold, at least about 8.5-fold, at least about 9.0-fold, at least about 9.5-fold, at least about 10.0-fold, at least about 10.5-fold, at least about 11.0-fold, at least about 11.5-fold, at least about 12.0-fold, at least about 12.5-fold, at least about 13.0-fold, at least about 13.5-fold, at least about 14.0-fold, at least about 14.5-fold, at least about 15.0-fold, at least about 16.0-fold, at least about 17.0-fold, at least about 18.0-fold, at least about 19.0-fold, at least about 20.0-fold, at least about 21.0-fold, at least about 22.0-fold, at least about 23.0-fold, at least about 24.0-fold, at least about 25.0-fold, at least about 26.0-fold, at least about 27.0-fold, at least about 28.0-fold, at least about 29.0-fold, or at least about 30.0-fold as compared to a subject not administered the immunogenic composition.

The induced cellular immune response can include an increased frequency of CD107a/IFNγ/T-bet triple-positive CD8 T cells that are reactive against the native antigen. The frequency of CD107a/IFNγ/T-bet triple-positive CD8 T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold as compared to a subject not administered the immunogenic composition.

The induced cellular immune response can include an increased frequency of CD107a/IFNγ double-positive CD8 T cells that are reactive against the native antigen. The frequency of CD107a/IFNγ double-positive CD8 T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, or 14-fold as compared to a subject not administered the immunogenic composition.

The cellular immune response induced by the immunogenic composition can include eliciting a CD4+ T cell response. The elicited CD4+ T cell response can be reactive with the native antigen genetically related to the optimized synthetic antigen. The elicited CD4+ T cell response can be polyfunctional. The induced cellular immune response can include eliciting a CD4+ T cell response, in which the CD4+ T cells produce IFN-γ, TNF-α, IL-2, or a combination of IFN-γ and TNF-α.

The induced cellular immune response can include an increased frequency of CD4+ T cells that produce IFN-γ. The frequency of CD4+IFN-γ+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, or 20-fold as compared to a subject not administered the immunogenic composition.

The induced cellular immune response can include an increased frequency of CD4+ T cells that produce TNF-α. The frequency of CD4+TNF-α+ T cells associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 11-fold, 12-fold, 13-fold, 14-fold, 15-fold, 16-fold, 17-fold, 18-fold, 19-fold, 20-fold, 21-fold, or 22-fold as compared to a subject not administered the immunogenic composition.

The induced cellular immune response can include an increased frequency of CD4+ T cells that produce both IFN-γ and TNF-α. The frequency of CD4+IFN-γ+TNF-α+ associated with the subject administered the immunogenic composition can be increased by at least about 2-fold, 2.5-fold, 3.0-fold, 3.5-fold, 4.0-fold, 4.5-fold, 5.0-fold, 5.5-fold, 6.0-fold, 6.5-fold, 7.0-fold, 7.5-fold, 8.0-fold, 8.5-fold, 9.0-fold, 9.5-fold, 10.0-fold, 10.5-fold, 11.0-fold, 11.5-fold, 12.0-fold, 12.5-fold, 13.0-fold, 13.5-fold, 14.0-fold, 14.5-fold, 15.0-fold, 15.5-fold, 16.0-fold, 16.5-fold, 17.0-fold, 17.5-fold, 18.0-fold, 18.5-fold, 19.0-fold, 19.5-fold, 20.0-fold, 21-fold, 22-fold, 23-fold 24-fold, 25-fold, 26-fold, 27-fold, 28-fold, 29-fold, 30-

fold, 31-fold, 32-fold, 33-fold, 34-fold, or 35-fold as compared to a subject not administered the immunogenic composition.

Other Components of the Composition

In some embodiments, the immunogenic composition of the invention further includes a pharmaceutically acceptable excipient. A pharmaceutically acceptable excipient can include such functional molecules as vehicles, adjuvants, carriers or diluents, which are known and readily available to the public. Preferably, the pharmaceutically acceptable excipient is an adjuvant or transfection facilitating agent. In some embodiments, the nucleic acid molecule, or DNA plasmid, is delivered to the cells in conjunction with administration of a polynucleotide function enhancer or a genetic vaccine facilitator agent (or transfection facilitating agent). Polynucleotide function enhancers are described in U.S. Serial Number 5,593,972, 5,962,428 and International Application Serial Number PCT/US94/00899 filed January 26, 1994, which are each incorporated herein by reference. Genetic vaccine facilitator agents are described in US. Serial Number 021,579 filed April 1, 1994, which is incorporated herein by reference. The transfection facilitating agent can be administered in conjunction with nucleic acid molecules as a mixture with the nucleic acid molecule or administered separately simultaneously, before or after administration of nucleic acid molecules. Examples of transfection facilitating agents includes surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the genetic construct. In some embodiments, the DNA plasmid vaccines may also include at least one transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid.

In some embodiments of the present invention, the immunogenic compositions can further include an adjuvant. In some embodiments, the adjuvant is selected from the group consisting of: alpha-interferon, gamma-interferon, platelet derived growth factor (PDGF), TNFα, TNFβ, GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated

epithelial chemokine (MEC), IL-12, IL-15, MHC, CD80, CD86 including IL-15 having the signal sequence deleted and optionally including the signal peptide from IgE. Other genes which may be useful adjuvants include those encoding: MCP-1, MIP-l-alpha, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, pl50.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, IkB, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof. In some preferred embodiments, the adjuvant is selected from IL-12, IL-15, CTACK, TECK, or MEC.

The immunogenic compositions according to the present invention are formulated according to the mode of administration to be used. In cases where DNA plasmid vaccines are injectable compositions, they are sterile, and/or pyrogen free and/or particulate free. An isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. In some embodiments, a vasoconstriction agent is added to the formulation. In some embodiments, a stabilizing agent that allows the formulation to be stable at room or ambient temperature for extended periods of time, such as LGS or other polycations or polyanions is added to the formulation.

The composition may further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient can be functional molecules such as vehicles, carriers, or diluents. The pharmaceutically acceptable excipient can be a transfection facilitating agent, which can include surface active agents, such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and the poly-L-glutamate may be present in the composition at a concentration less than 6 mg/ml. The transfection facilitating agent may also include surface active agents such as immune-stimulating complexes (ISCOMS), Freunds incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid may also be used administered in conjunction with the composition. The composition may also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640), calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than 0.050 mg/ml, or less than 0.010 mg/ml.

The composition may further comprise a genetic facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

The composition may comprise nucleic acid at quantities of from about 1 nanogram to 100 milligrams; about 1 microgram to about 10 milligrams; or preferably about 0.1 microgram to about 10 milligrams; or more preferably about 1 milligram to about 2 milligrams. In some preferred embodiments, composition according to the present invention comprises about 5 nanograms to about 1000 micrograms of nucleic acid. In some preferred embodiments, composition can contain about 10 nanograms to about 800 micrograms of nucleic acid. In some preferred embodiments, the composition can contain about 0.1 to about 500 micrograms of nucleic acid. In some preferred embodiments, the composition can contain about 1 to about 350 micrograms of nucleic acid. In some preferred embodiments, the composition can contain about 25 to about 250 micrograms, from about 100 to about 200 microgram, from about 1 nanogram to 100 milligrams; from about 1 microgram to about 10 milligrams; from about 0.1 microgram to about 10 milligrams; from about 1 milligram to about 2 milligram, from about 5 nanograms to about 1000 micrograms, from about 10 nanograms to about 800 micrograms, from about 0.1 to about 500 micrograms, from about 1 to about 350 micrograms, from about 25 to about 250 micrograms, from about 100 to about 200 microgram of nucleic acid.

The composition can be formulated according to the mode of administration to be used. An injectable pharmaceutical composition can be sterile, pyrogen free and particulate free. An isotonic formulation or solution can be used. Additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol, and lactose. The composition can comprise a vasoconstriction agent. The isotonic solutions can include phosphate buffered saline. The composition can further comprise stabilizers including gelatin and albumin. The stabilizers can allow the formulation to be stable at room or ambient temperature for extended periods of time, including LGS or polycations or polyanions.

Methods of Delivery of the Composition

Another aspect of the present invention provides methods of eliciting an immune response against one or more ASFV virus in a mammal, comprising delivering an immunogenic composition to tissue of the mammal, the immunogenic composition comprising at least one nucleic acid molecule capable of expressing a synthetic antigen of the one or more ASFV virus in a cell of the mammal to elicit an immune response in the mammal.

The present invention also relates to methods of delivering the composition to the subject in need thereof. The method of delivery can include, administering the composition to the subject. Administration can include, but is not limited to, DNA injection with and without in vivo electroporation, liposome mediated delivery, and nanoparticle facilitated delivery.

The mammal receiving delivery of the composition may be a member of the pig family, including domesticated swine, wild boars, feral swine, warthogs, bush pigs, and giant forest hogs.

The composition may be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The composition may be administered by traditional syringes, needleless

injection devices, “microprojectile bombardment gone guns”, or other physical methods such as electroporation (“EP”), “hydrodynamic method”, or ultrasound.

Electroporation

Administration of the composition via electroporation may be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal, a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device may comprise an electroporation component and an electrode assembly or handle assembly. The electroporation component may include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation may be accomplished using an in vivo electroporation device, for example CELLECTRA EP system (Inovio Pharmaceuticals, Plymouth Meeting, PA) or Elgen electroporator (Inovio Pharmaceuticals, Plymouth Meeting, PA) to facilitate transfection of cells by the plasmid.

The electroporation component may function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component may function as more than one element of the electroporation devices, which may be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device may not be limited, as the elements can function as one device or as separate elements in communication with one another. The electroporation component may be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly may include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism may receive the measured impedance

and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes may deliver the pulse of energy in a decentralized pattern. The plurality of electrodes may deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence may comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism may be performed by either hardware or software. The feedback mechanism may be performed by an analog closed-loop circuit. The feedback occurs every 50 µs, 20 µs, 10 µs or 1 µs, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode may measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism may maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

Examples of electroporation devices and electroporation methods that may facilitate delivery of the composition of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that may be used for facilitating delivery of the composition include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems may comprise a plurality of needle

electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which may be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 may be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004,

which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

Method of Treatment

Also provided herein is a method of treating, protecting against, and/or preventing disease in a subject in need thereof by inducing an immune response against a viral antigen in the subject. In certain embodiments, the invention provides a method of treating, protecting against, and/or preventing at least one of an ASFV virus infection or an ASFV associated pathology in a subject. In one embodiment, an ASFV associated pathology is lethal hemorrhagic fever.

The method can include administering an immunogenic composition of the invention to the subject. Administration of the composition to the subject can be done using the method of delivery described above.

The composition dose can be between 1 μg to 10 mg active component/kg body weight/time, and can be 20 μg to 10 mg component/kg body weight/time. The composition can be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days. The number of composition doses for effective treatment can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

Combination Vaccine

The present invention also provides a method of treating, protecting against, and/or preventing disease in a subject in need thereof by administering a combination of two or more nucleic acid molecules or immunogenic compositions wherein each of the two or more nucleic acid molecules or immunogenic compositions encodes an optimized synthetic viral antigen.

The two or more nucleic acid molecules or immunogenic compositions may be administered using any suitable method such that a combination of two or more nucleic acid molecules or immunogenic compositions are both present in the subject. In one embodiment, the method may comprise administration of a first nucleic acid molecule or immunogenic composition of the invention by any of the methods described in detail above and administration of a second nucleic acid molecule or immunogenic composition less than 1, less than 2, less than 3, less than 4, less than 5, less than 6, less than 7, less than 8, less than

9 or less than 10 days following administration of the first nucleic acid molecule or immunogenic composition of the invention. In one embodiment, the method may comprise administration of at least 2, at least 3, at least 4, at least 5, at least 6 or more than 6 nucleic acid molecules or immunogenic compositions concurrently at different sites on the same subject. In one embodiment, the method may comprise administration of at least 2, at least 3, at least 4, at least 5, at least 6 or more than 6 nucleic acid molecules or immunogenic compositions more than 1, more than 2, more than 3, more than 4, more than 5, more than 6, more than 7, more than 8, more than 9 or more than 10 days following administration of a first nucleic acid molecule or immunogenic composition. In one embodiment, the method may comprise administration of at least 2, at least 3, at least 4, at least 5, at least 6 or more than 6 nucleic acid molecules or immunogenic compositions less than 1, less than 2, less than 3, less than 4, less than 5, less than 6, less than 7, less than 8, less than 9 or less than 10 days following administration of a first nucleic acid molecule or immunogenic composition.

EXAMPLES

The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Preferably the DNA formulations for use with a muscle or skin EP device described herein have high DNA concentrations, preferably concentrations that include microgram to tens of milligram quantities, and preferably milligram quantities, of DNA in small volumes that are optimal for delivery to the skin, preferably small injection volume, ideally 25-200 microliters (μL). In some embodiments, the DNA formulations have high DNA concentrations, such as 1 mg/mL or greater (mg DNA/volume of formulation). More

preferably, the DNA formulation has a DNA concentration that provides for gram quantities of DNA in 200 μL of formula, and more preferably gram quantities of DNA in 100 μL of formula.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in US application no. 12/126611 which published as US Publication No. 20090004716 on January 1, 2009. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in US Publication No. 20090004716 and those described in US Patent No. 7,238,522, which issued on July 3, 2007. The high concentrations of plasmids used with the skin EP devices and delivery techniques described herein allow for administration of plasmids into the ID/SC space in a reasonably low volume and aids in enhancing expression and immunization effects. The publications, US Publication No. 20090004716 and US Patent No. 7,238,522, are hereby incorporated in their entirety.

Example 1: Construction & characterization of immune responses to novel Synthetic DNA vaccines against African swine fever Virus

4 cassettes for were developed for a study covering antigens reported to be involved in generation of protection. The goal is to drive strong T cell immunity. Targets were p32, p54, CD2 and p12. Comparisons with ubiquitin targeting sequences were included. An improved synthetic IgE leader sequence was included for both increased expression as well as immune targeting. All constructs were studied for expression in vitro in cells: examples provided. Specific antisera was generated in a pilot immunization study for follow antigen expression.

The data presented herein demonstrates that synthetic enhanced vaccines (SEV) against ASFV were developed that are simple to administer and immunogenic in vivo. Several ASFV-Antigen encoding vaccines were immunogenic driving seroconversion in all immunized animals, showing vaccine consistency. Immunization with ASFV-p32, p54 and CD2 resulted in induction of strong cellular and humoral responses. The most potent T cell responses were observed to CD2 antigen. The results further indicate that there were superior

or equivalent serology responses combined with superior T cell responses induced by construct 3 (ASFV-ubiquitin+CD2-p32+p54).

The materials and methods used, and the results are now described

Plasmid vaccine constructions

Constructs were designed and optimized for use. An optimized leader sequence was included (Figure 1).

Animals and vaccinations

Balb/C mice were immunized with a prime on day 0, and boost at day 21, & 28. Analysis was performed one-week post 3rd immunization (Figure 2). Immunizations were administered using Inovio Adaptive Electroporation (Facilitated Delivery) driving enhanced plasmid vaccine uptake in vivo. Sera was collected for immune analysis against target cells expressing ASFV antigens (Figure 3-Figure 5).

Serology

Sera was isolated from immunized mice as indicated vaccine groups and binding IgG levels specific for linear epitopes against ASFV-p32 (15mer peptides overlapping by 11 amino acids spanning the entire ASFV-p32 sequence were mixed into one pools) and were evaluated by peptide ELISA. Data shown is the average from eight mice for the groups 2 & 4 and ten mice for the group 1 & 3 immunized with 25μg ASFV synthetic vaccine groups (Figure 7-Figure 10).

Cellular response

Groups of mice (n#5/group) were immunized three times, each 2 weeks apart with 25μg of vaccines. Samples were collected a week after the third immunization. Target - specific CD8" T-lymphocyte responses were assessed by IFN-γ ELISpot assays to a peptide pool covering the indicated protein. Mean responses were measured in each groups one week after the third immunization (Figure 11). Error bars indicate standard errors. Responses to p32 antigen were similar across the 4 designs, with design 2 being slightly favored. Responses to p54 were relatively similar across designs with design 3 being slightly favored.

Only groups 2 and 3 drove responses to CD2, and these were the highest responders. Only group 4 developed low T cell responses to p12.

Intracellular Cytokine Staining Assay

Mice were injected with DNA encoding CD2-p32+p54 (Figure 12) or Ubi+CD2-p32+p54 (Figure 13), and intracellular cytokine staining (ICS) was determined. For both constructs, on average overall, the majority of CD4+ /CD8+ T cells produced all three cytokines assessed (IFN-g, IL-2, and TNF-a) (Figure 12 and Figure 13). In addition, both constructs demonstrate poly-functionality.

It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the invention, may be made without departing from the spirit and scope thereof.

CLAIMS

What is claimed:

1. A nucleic acid molecule comprising a nucleotide sequence encoding at least one synthetic African swine fever virus (ASFV) antigen.

2. The nucleic acid molecule of claim 1 comprising at least one nucleotide sequence encoding a peptide comprising an amino acid sequence selected from the group consisting of

a) an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30,

b) an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30,

c) the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30, and

d) an immunogenic fragment comprising at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30.

3. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule is selected from the group consisting of a DNA molecule and an RNA molecule.

4. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule comprises at least one nucleotide sequence selected from the group consisting of

a) a nucleotide sequence having at least about 90% identity over an entire length of a nucleotide sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 and SEQ ID NO:29,

b) an immunogenic fragment of a nucleotide sequence having at least about 90% identity over at least 60% of the nucleotide sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 and SEQ ID NO:29,

c) a nucleotide sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 and SEQ ID NO:29, and

d) an immunogenic fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:21, SEQ ID NO:23 and SEQ ID NO:29.

5. The nucleic acid molecule of claim 1, wherein the encoded peptide is operably linked to at least one regulatory sequence selected from the group consisting of a start codon, an IgE leader sequence and a stop codon.

6. The nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes at least two peptides comprising an amino acid sequence selected from the group consisting of

a) an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30,

b) an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30,

c) the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30, and

d) an immunogenic fragment comprising at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30.

7. The nucleic acid molecule of claim 6, wherein the nucleic acid molecule encodes an amino acid sequence selected from the group consisting of

a) an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20,

b) an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20,

c) the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ

ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20, and

d) an immunogenic fragment comprising at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20.

8. The nucleic acid molecule of claim 7, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of

a) a nucleotide sequence having at least about 90% identity over an entire length of a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19,

b) an immunogenic fragment of a nucleotide sequence having at least about 90% identity over at least 60% of the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19,

c) a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19, and

d) an immunogenic fragment of a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:19.

9. The nucleic acid molecule of any one of claims 1-8, wherein the nucleic acid molecule comprises an expression vector.

10. The nucleic acid molecule of any one of claims 1-8, wherein the nucleic acid molecule comprises a viral particle.

11. An immunogenic composition comprising at least one nucleic acid molecule of any one of claims 1-10.

12. The immunogenic composition of claim 11, further comprising a pharmaceutically acceptable excipient.

13. The immunogenic composition of claim 11, further comprising an adjuvant.

14. A peptide comprising at least one amino acid sequence selected from the group consisting of

a) an amino acid sequence having at least about 90% identity over an entire length of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30,

b) an immunogenic fragment comprising at least about 90% identity over at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30,

c) the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30, and

d) an immunogenic fragment comprising at least 60% of the amino acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:22, SEQ ID NO:24 and SEQ ID NO:30.

15. An immunogenic composition comprising a peptide of claim 14.

16. A method of inducing an immune response against an ASFV antigen in a subject in need thereof, the method comprising administering a nucleic acid molecule of any one of claims 1-10 or the immunogenic composition of any one of claims 11-13 to the subject.

17. The method of claim 16, wherein administering includes at least one of electroporation and injection.

18. A method of treating or preventing an ASFV associated pathology in subject in need thereof, the method comprising administering a nucleic acid molecule of any one of claims 1-10 or the immunogenic composition of any one of claims 11-13 to the subject.

19. The method of claim 18, wherein administering includes at least one of electroporation and injection.

20. The method of claim 18, wherein the ASFV associated pathology is lethal hemorrhagic fever.