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Jarnigan Drive, Athens, GA 30605 (US). **BUBLOT, Michel** [BE/FR]; 3 Rue Favre Garin, F-69630 Chaponost (FR). **COX, Kevin** [US/US]; 1916 Sunset Drive, Raleigh, NC 27608 (US).

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(74) Agent: **JARECKI-BLACK, Judy**; 3239 Satellite Blvd., Duluth, GA 30096 (US).

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(71) Applicants (for all designated States except US): **MERIAL LIMITED** [US/US]; 3239 Satellite Blvd., Duluth, GA 30096 (US). **BIOLEX THERAPEUTICS** [US/US]; 158 Credle Street, Pittsboro, NC 27312 (US).

(72) Inventors; and

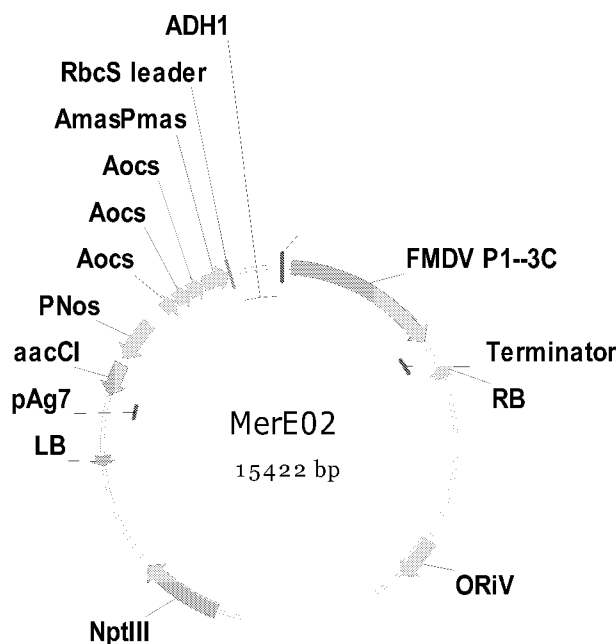
(75) Inventors/Applicants (for US only): **AUDONNET, Jean-Christophe** [FR/FR]; 119, Rue De Crequi, F-69006 Lyon (FR). **GUO, Xuan** [CN/US]; 233 Reynoldston Way, Suwanee, GA 30024 (US). **FEILMEIER, Bradley, J.** [US/US]; 1170 Windy Creek Drive, Watkinsville, GA 30677 (US). **TROUPE, Karolyn, Marie** [US/US]; 166

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(54) Title: FOOT AND MOUTH DISEASE VIRUS RECOMBINANT VACCINES AND USES THEREOF

(57) Abstract: The present invention encompasses FMDV vaccines or compositions. The vaccine or composition may be a vaccine or composition containing FMDV antigens. The invention also encompasses recombinant vectors encoding and expressing FMDV antigens, epitopes or immunogens which can be used to protect animals, in particular ovines, bovines, caprines, or porcines, against FMDV.



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**FOOT AND MOUTH DISEASE VIRUS RECOMBINANT VACCINES AND USES  
THEREOF**

**CROSS-REFERENCE TO RELATED APPLICATIONS**

5           This application claims benefit of US provisional application Serial No. 61/313,164 filed March 12, 2010 and US provisional application Serial No. 61/366,363 filed July 21, 2010.

**FIELD OF THE INVENTION**

10           The present invention relates to compositions for combating Foot and Mouth Disease Virus (FMDV) infection in animals. The present invention provides pharmaceutical compositions comprising an FMDV antigen, methods of vaccination against FMDV, and kits for use with such methods and compositions.

**BACKGROUND OF THE INVENTION**

15           Foot-and-mouth disease (FMD) is one of the most virulent and contagious diseases affecting farm animals. This disease is endemic in numerous countries in the world, especially in Africa, Asia and South America. In addition, epidemic outbreaks can occur periodically. The presence of this disease in a country may have very severe economic consequences resulting from loss of productivity, loss of weight and milk production in infected herds, and from trade embargoes imposed on these countries. The measures taken  
20           against this disease consist of strict application of import restrictions, hygiene controls and quarantine, slaughtering sick animals and vaccination programs using inactivated vaccines, either as a preventive measure at the national or regional level, or periodically when an epidemic outbreak occurs.

25           FMD is characterized by its short incubation period, its highly contagious nature, the formation of ulcers in the mouth and on the feet and sometimes, the death of young animals. FMD affects a number of animal species, in particular cattle, pigs, sheep and goats. The agent responsible for this disease is a ribonucleic acid (RNA) virus belonging to the Aphthovirus genus of the *Picornaviridae* family (Cooper *et al.*, Intervirology, 1978, 10, 165-180). At present, at least seven types of foot-and-mouth disease virus (FMDV) are known: the  
30           European types (A, O and C), the African types (SAT1, SAT2 and SAT3) and an Asiatic type (Asia 1). Numerous sub-types have also been distinguished (Kleid *et al.* Science (1981), 214, 1125-1129).

FMDV is a naked icosahedral virus of about 25 nm in diameter, containing a single-stranded RNA molecule consisting of about 8500 nucleotides, with a positive polarity. This RNA molecule comprises a single open reading frame (ORF), encoding a single polyprotein containing, *inter alia*, the capsid precursor also known as protein P1 or P88. The protein P1 is  
5 myristylated at its amino-terminal end. During the maturation process, the protein P1 is cleaved by the protease 3C into three proteins known as VP0, VP1 and VP3 (or 1AB, 1D and 1C respectively; Belsham G. J., Progress in Biophysics and Molecular Biology, 1993, 60, 241-261). In the virion, the protein VP0 is then cleaved into two proteins, VP4 and VP2 (or 1A and 1B respectively). The mechanism for the conversion of the proteins VP0 into VP4  
10 and VP2, and for the formation of mature virions is not known. The proteins VP1, VP2 and VP3 have a molecular weight of about 26,000 Da, while the protein VP4 is smaller at about 8,000 Da.

The simple combination of the capsid proteins forms the protomer or 5S molecule, which is the elementary constituent of the FMDV capsid. This protomer is then complexed  
15 into a pentamer to form the 12S molecule. The virion results from the encapsidation of a genomic RNA molecule by assembly of twelve 12S pentamers, thus constituting the 146S particles. The viral capsid may also be formed without the presence of an RNA molecule inside it (hereinafter "empty capsid"). The empty capsid is also designated as particle 70S. The formation of empty capsids may occur naturally during viral replication or may be  
20 produced artificially by chemical treatment.

Many hypotheses, research routes, and proposals have been developed in an attempt to design effective vaccines against FMD. Currently, the only vaccines on the market comprise inactivated virus. Concerns about safety of the FMDV vaccine exist, as outbreaks of FMD in Europe have been associated with shortcomings in vaccine manufacture (King, A.M.Q. *et al*, (1981) Nature 293: 479-480). The inactivated vaccines do not confer long-term  
25 immunity, thus requiring booster injections given every year, or more often in the event of epidemic outbreaks. In addition, there are risks linked to incomplete inactivation and/or to the escape of virus during the production of inactivated vaccines (King, A.M.Q., *ibid*). A goal in the art has been to construct conformationally correct immunogens lacking the infective  
30 FMDV genome to make effective and safe vaccines.

Vaccinia virus has been used successfully to immunize against smallpox, culminating in the worldwide eradication of smallpox in 1980. Thus, a new role for poxviruses became important, that of a genetically engineered vector for the expression of foreign genes

(Panicali and Paoletti, 1982; Paoletti *et al.*, 1984). Genes encoding heterologous antigens have been expressed in vaccinia, often resulting in protective immunity against challenge by the corresponding pathogen (reviewed in Tartaglia *et al.*, 1990). A highly attenuated strain of vaccines, designated MVA, has also been used as a vector for poxvirus-based vaccines. Use  
5 of MVA is described in U.S. Patent No. 5,185,146.

Additional vaccine vector systems involve the use of avipox viruses, which are naturally host-restricted poxviruses. Both fowlpoxvirus (FPV; Taylor *et al.* 1988a, b) and canarypoxvirus (CPV; Taylor *et al.*, 1991 & 1992) have been engineered to express foreign gene products. Fowlpox virus (FPV) is the prototypic virus of the Avipox genus of the  
10 Poxvirus family. The virus causes an economically important disease of poultry that has been well controlled since the 1920's by the use of live attenuated vaccines. Replication of the avipox viruses is limited to avian species (Matthews, 1982) and there are no reports in the literature of avipox virus causing a productive infection in any non-avian species including  
15 man. This host restriction provides an inherent safety barrier against transmission of the virus to other species and makes the use of avipox virus based vaccine vectors in veterinary and human applications an attractive proposition.

Other attenuated poxvirus vectors have been prepared by genetic modifications of wild type strains of virus. The NYVAC vector, derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia *et al.*, 1992) has proven  
20 useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen. Another engineered poxvirus vector is ALVAC, derived from canarypox virus (see U.S. Patent No. 5,756,103). ALVAC does not productively replicate in non-avian hosts, a characteristic thought to improve its safety profile (Taylor *et al.*, 1991 & 1992). ALVAC was deposited under the terms of the Budapest Treaty with the American Type  
25 Culture Collection under accession number VR-2547. Yet another engineered poxvirus vector is TROVAC, derived from fowlpox virus (see U.S. Patent No. 5,766,599).

Recombinant poxviruses can be constructed in two steps known in the art and analogous to the methods for creating synthetic recombinants of poxviruses such as the vaccinia virus and avipox virus described in U.S. Patent Nos. 4,769,330; 4,722,848;  
30 4,603,112; 5,110,587; 5,174,993; 5,494,807; and 5,505,941, the disclosures of which are incorporated herein by reference. It can thus be appreciated that provision of an FMDV recombinant poxvirus, and of compositions and products therefrom, particularly ALVAC or TROVAC-based FMDV recombinants and compositions and products therefrom, especially

such recombinants containing the P1 genes and/or 3C protease gene of FMDV, and compositions and products therefrom, would be a highly desirable advance over the current state of technology.

5 Recently, plants have been investigated as a source for the production of therapeutic agents such as vaccines, antibodies, and biopharmaceuticals. However, the production of vaccines, antibodies, proteins, and biopharmaceuticals from plants is far from a remedial process, and there are numerous obstacles that are commonly associated with such vaccine production. Limitations to successfully producing plant vaccines include low yield of the bioproduct or expressed antigen (Chargelegue et al., Trends in Plant Science 2001, 6, 495-10 496), protein instability, inconsistencies in product quality (Schillberg et al., Vaccine 2005, 23, 1764-1769), and insufficient capacity to produce viral-like products of expected size and immunogenicity (Arntzen et al., Vaccine 2005, 23, 1753-1756). In order to address these problems, codon optimization, careful approaches to harvesting and purifying plant products, use of plant parts such as chloroplasts to increase uptake of the material, and improved subcellular targeting are all being considered as potential strategies (Koprowski, Vaccine 15 2005, 23, 1757-1763).

Considering the susceptibility of animals (including humans, albeit rarely), to FMDV, a method of preventing FMDV infection and protecting animals is essential. Accordingly, there is a need for an effective vaccine against FMDV.

20

### **SUMMARY OF THE INVENTION**

Compositions comprising an antigenic FMDV polypeptide and fragments and variants thereof are provided. The FMDV antigens and fragments and variants thereof possess immunogenic and protective properties. The FMDV antigens may be produced in a plant or 25 algae.

The antigenic polypeptides and fragments and variants thereof can be formulated into vaccines and/or pharmaceutical compositions. Such vaccines can be used to vaccinate an animal and provide protection against at least one FMDV strain.

30 Methods of the invention include methods for making the antigenic polypeptides in duckweed plant. Methods also include methods of use including administering to an animal an effective amount of an antigenic polypeptide or fragment or variant thereof to produce a protective immunogenic response. After production in duckweed the antigenic polypeptide can be partially or substantially purified for use as a vaccine.

Kits comprising at least one antigenic polypeptide or fragment or variant thereof and instructions for use are also provided.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

5           The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings, in which:

          FIG. 1 depicts a table summarizing the DNA and Protein sequences, presented as a listing in the appendix of the instant application;

10           FIG. 2 represents the native and optimized FMDV sequence that was expressed in duckweed. The expressed polypeptide is cleaved into its individual proteins by the 3C protease that folds after translation, self-cleaves to release itself from the polypeptide, and finally perform internal cleavages within the P1 polypeptide.

          FIG. 3 depicts the identity and placement of the duckweed-optimized FMDV antigens  
15 for the 4 “MerE” duckweed expression constructs;

          FIG. 4 is a sequence alignment of FMDV optimized for expression in duckweed (SEQ ID NO:2) and FMDV optimized for expression in mammalian cells (SEQ ID NO:1). The sequence identity is indicated;

          FIG. 5 depicts the pHM-1119-1 plasmid, which contains nucleic acid sequence  
20 encoding the mammalian optimized FMDV P1-3C (SEQ ID NO:1);

          FIG. 6 depicts the pMerE01 plasmid, Strong Promoter + P1+2A/2B1 (A24) + 3C (A12);

          FIG. 7 depicts the pMerE02, Strong Promoter + P1;

          FIG. 8 depicts the pMerE03 plasmid, Strong Promoter + P1+2A/2B1 (A24) + Weak  
25 Promoter 3C (A12);

          FIG.9 depicts the pMerE04 plasmid, Strong Promoter + P1+2A/2B1 (A24) + Weak Promoter 3C (A12) with optimized 5’UTR;

          FIG. 10 depicts a representative RNA dot blot used to screen recombinant duckweed cell lines for expression of genes encoding FMDV antigens;

30           FIG. 11 depicts a representative quantitative PCR results for duckweed cell lines expressing FMDV constructs;

          FIG. 12 depicts the Western blot for duckweed cell lines harboring and expressing MerE01 and MerE02;

FIG. 13 depicts electron micrographs of inventive FMDV virus like particles (VLP);

FIG. 14 depicts electron micrograph of clusters of FMDV VLP;

FIG. 15 depicts WB Analysis of MerE01 and MerE03 Crude Extract (5ug TSP/lane) using Guinea Pig Serum. For MerE01, VP1 (VP3) band(s) observed, suggesting expression  
5 of both P1 and 3C and further processing of P1. For MerE03, neither P1 nor VP1 (VP3) were observed, suggesting expression of 3C and degradation of P1;

FIG. 16 presents an FMD viral particle schematic diagram;

FIG. 17 presents OD of decreasing concentrations of various extracts from duckweed expressing FMDV antigens as measured by ELISA;

10 FIG. 18 presents MerF01 plasmid map with feature summary table;

FIG. 19 presents MerF02 plasmid map with feature summary table;

FIG. 20 presents MerF03 plasmid map with feature summary table;

FIG. 21 presents MerF04 plasmid map with feature summary table;

FIG. 22 presents MerF05 plasmid map with feature summary table;

15 FIG. 23 presents MerF06 plasmid map with feature summary table.

#### **DETAILED DESCRIPTION**

Compositions comprising an FMDV polypeptide, antigen and fragments and variants thereof that elicit an immunogenic response in an animal are provided. The antigenic polypeptides or fragments or variants thereof are produced in a plant or algae. The antigenic  
20 polypeptides or fragments or variants may be formulated into vaccines or pharmaceutical compositions and used to elicit or stimulate a protective response in an animal. In one embodiment the polypeptide antigen is an FMDV P1 or 3C polypeptide or active fragment or variant thereof.

It is recognized that the antigenic polypeptides of the invention may be full length  
25 polypeptides or active fragments or variants thereof. By “active fragments” or “active variants” is intended that the fragments or variants retain the antigenic nature of the polypeptide. Thus, the present invention encompasses any FMDV polypeptide, antigen, epitope or immunogen that elicits an immunogenic response in an animal. The FMDV polypeptide, antigen, epitope or immunogen may be any FMDV polypeptide, antigen, epitope  
30 or immunogen, such as, but not limited to, a protein, peptide or fragment or variant thereof, that elicits, induces or stimulates a response in an animal, such as an ovine, bovine, caprine or porcine.



Particular FMDV antigenic polypeptides include P1 and 3C. FMDV is a non-enveloped icosahedral virus of about 25 nm in diameter, containing a single-stranded RNA molecule consisting of about 8500 nucleotides, with a positive polarity. This RNA molecule comprises a single open reading frame (ORF), encoding a single polyprotein containing, *inter alia*, the capsid precursor also known as protein P1 or P88. The protein P1 is myristylated at its amino-terminal end. During the maturation process, the protein P1 is cleaved by the protease 3C into three proteins known as VP0, VP1 and VP3 (or 1AB, 1D and 1C respectively; Belsham G. J., *Progress in Biophysics and Molecular Biology*, 1993, 60, 241-261). In the virion, the protein VP0 is then cleaved into two proteins, VP4 and VP2 (or 1A and 1B respectively). The mechanism for the conversion of the proteins VP0 into VP4 and VP2, and for the formation of mature virions is not known. The proteins VP1, VP2 and VP3 have a molecular weight of about 26,000 Da, while the protein VP4 is smaller at about 8,000 Da. FMDV sequences are also described in US Patent numbers US 7,527,960 and US 7,531,182, which documents are herein incorporated in their entirety.

The, simple combination of the capsid proteins forms the protomer or 5S molecule, which is the elementary constituent of the FMDV capsid. This protomer is then complexed into a pentamer to form the 12S molecule. The virion results from the encapsidation of a genomic RNA molecule by assembly of twelve 12S pentamers, thus constituting the 146S particles. The viral capsid may also be formed without the presence of an RNA molecule inside it (hereinafter "empty capsid"). The empty capsid is also designated as particle 70S. The formation of empty capsids may occur naturally during viral replication or may be produced artificially by chemical treatment.

The present invention relates to bovine, ovine, caprine, or porcine vaccines or compositions which may comprise an effective amount of a recombinant FMDV antigen and a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle.

In some embodiments, the vaccines further comprise adjuvants, such as the oil-in-water (O/W) emulsions described in US Patent 7371395.

In still other embodiments, the adjuvants include EMULSIGEN, Aluminum Hydroxide, Saponin, and CpG, or combinations thereof.

In some embodiments, the response in the animal is a protective immune response.

By "animal" it is intended mammals, birds, and the like. Animal or host includes mammals and human. The animal may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic

cats, wild cats, other big cats, and other felines including cheetahs and lynx), ovine (e.g., sheep), bovine (e.g., cattle), porcine (e.g., pig), caprine (e.g., goat), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow, ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), and fish. The term “animal” also  
5 includes an individual animal in all stages of development, including embryonic and fetal stages.

The term “plants” as used herein includes both dicotyledonous (dicot) plants and monocotyledonous (monocot) plant. Dicot plants include, but are not limited to, legumes such as pea, alfalfa and soybean, carrot, celery, tomato, potato, tobacco, pepper, oilseed rape,  
10 beet, cabbage, cauliflower, broccoli, lettuce, peanut, and the like. Monocot plants include, but are not limited to, cereals such as wheat, barley, sorghum and millet, rye, triticale, maize, rice or oats, sugarcane, members of the microalgae family, grasses, and the like. The term “plant” also include non-flowering plants including, but not limited to, ferns, horsetails, club mosses, mosses, liverworts, hornworts, algae, for example, red, brown, and green algae,  
15 gametophytes, and the like.

The term “algae” and “alga” as used herein includes any strain of algae capable of producing a polypeptide or fragment or variant thereof. The algae may be microalgae. The microalgae may be *Thraustochytriaceae*, for example, *Schizochytrium*, *Thraustochytrium*, *Labyrinthuloides*, and *Japonochytrium*.

20 Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms “a”, “an”, and “the” include plural referents unless context clearly indicates otherwise. Similarly, the word “or” is intended to include “and” unless the context clearly indicate otherwise.

25 It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as “comprises”, “comprised”, “comprising” and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean “includes”, “included”, “including”, and the like; and that terms such as “consisting essentially of” and “consists essentially of” have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not  
30 explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

The antigenic polypeptides of the invention are capable of protecting against FMDV. That is, they are capable of stimulating an immune response in an animal. By “antigen” or

“immunogen” means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert with immunogenic properties; a piece or fragment of DNA capable of inducing an immune response upon presentation to a host animal; a polypeptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

The term “immunogenic protein, polypeptide, or peptide” as used herein includes polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the total protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic determinant. An “immunogenic” protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By “immunogenic fragment” is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. See, e.g., Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996). For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Pat. No. 4,708,871; Geysen et al., 1984; Geysen et al., 1986. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, supra. Methods especially applicable to the proteins of *T. parva* are fully described in PCT/US2004/022605 incorporated herein by reference in its entirety.

As discussed the invention encompasses active fragments and variants of the antigenic polypeptide. Thus, the term “immunogenic protein, polypeptide, or peptide” further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. The term “conservative variation” denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence

such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) 5 basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine 10 for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference 15 molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted 20 polypeptide also immunoreact with the unsubstituted polypeptide.

The term "epitope" refers to the site on an antigen or hapten to which specific B cells and/or T cells respond. The term is also used interchangeably with "antigenic determinant" or "antigenic determinant site". Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another 25 antibody to a target antigen.

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T 30 cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of

symptoms normally displayed by an infected host, a quicker recovery time and/or a lowered viral titer in the infected host.

Synthetic antigens are also included within the definition, for example, polypeptides, flanking epitopes, and other recombinant or synthetically derived antigens. See, e.g.,  
5 Bergmann et al., 1993; Bergmann et al., 1996; Suhrbier, 1997; Gardner et al., 1998. Immunogenic fragments, for purposes of the present invention, will usually include at least about 3 amino acids, at least about 5 amino acids, at least about 10-15 amino acids, or about 15-25 amino acids or more amino acids, of the molecule. There is no critical upper limit to the length of the fragment, which could comprise nearly the full-length of the protein  
10 sequence, or even a fusion protein comprising at least one epitope of the protein.

Accordingly, a minimum structure of a polynucleotide expressing an epitope is that it comprises or consists essentially of or consists of nucleotides encoding an epitope or antigenic determinant of an FMDV polypeptide. A polynucleotide encoding a fragment of an FMDV polypeptide may comprise or consist essentially of or consist of a minimum of 15  
15 nucleotides, about 30-45 nucleotides, about 45-75, or at least 57, 87 or 150 consecutive or contiguous nucleotides of the sequence encoding the polypeptide. Epitope determination procedures, such as, generating overlapping peptide libraries (Hemmer et al., 1998), Pepscan (Geysen et al., 1984; Geysen et al., 1985; Van der Zee R. et al., 1989; Geysen, 1990; Multipin. RTM. Peptide Synthesis Kits de Chiron) and algorithms (De Groot et al., 1999;  
20 PCT/US2004/022605) can be used in the practice of the invention.

The term "nucleic acid" and "polynucleotide" refers to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant  
25 polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars and linking groups such as fluororibose and thiolate, and nucleotide branches. The sequence of nucleotides may be further modified after polymerization, such as  
30 by conjugation, with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions,

labeling components, other polynucleotides or solid support. The polynucleotides can be obtained by chemical synthesis or derived from a microorganism.

The term “gene” is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes include introns and exons as in genomic sequence, or  
5 just the coding sequences as in cDNAs and/or the regulatory sequences required for their expression. For example, gene also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

The invention further comprises a complementary strand to a polynucleotide encoding an FMDV antigen, epitope or immunogen. The complementary strand can be polymeric and  
10 of any length, and can contain deoxyribonucleotides, ribonucleotides, and analogs in any combination.

The terms “protein”, “peptide”, “polypeptide” and “polypeptide fragment” are used interchangeably herein to refer to polymers of amino acid residues of any length. The polymer can be linear or branched, it may comprise modified amino acids or amino acid  
15 analogs, and it may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling or bioactive component.

An “isolated” biological component (such as a nucleic acid or protein or organelle) refers to a component that has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been “isolated” include nucleic acids and  
20 proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant technology as well as chemical synthesis.

The term “purified” as used herein does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide preparation is one in which the polypeptide is more enriched than the polypeptide is in its natural environment.  
30 That is the polypeptide is separated from cellular components. By “substantially purified” it is intended that such that the polypeptide represents several embodiments at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, or more of the cellular components or materials have been removed. Likewise, the polypeptide may be partially

purified. By “partially purified” is intended that less than 60% of the cellular components or material is removed. The same applies to polynucleotides. The polypeptides disclosed herein can be purified by any of the means known in the art.

As noted above, the antigenic polypeptides or fragments or variants thereof are  
5 FMDV antigenic polypeptides that are produced in duckweed. Fragments and variants of the disclosed polynucleotides and polypeptides encoded thereby are also encompassed by the present invention. By “fragment” is intended a portion of the polynucleotide or a portion of the antigenic amino acid sequence encoded thereby. Fragments of a polynucleotide may encode protein fragments that retain the biological activity of the native protein and hence  
10 have immunogenic activity as noted elsewhere herein. Fragments of the polypeptide sequence retain the ability to induce a protective immune response in an animal.

“Variants” is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a deletion and/or addition of one or more nucleotides at one or more sites within the native polynucleotide and/or a substitution of one or more nucleotides at one or  
15 more sites in the native polynucleotide. As used herein, a “native” polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively. Variants of a particular polynucleotide of the invention (i.e., the reference polynucleotide) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by  
20 the reference polynucleotide. “Variant” protein is intended to mean a protein derived from the native protein by deletion or addition of one or more amino acids at one or more sites in the native protein and/or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they the ability to elicit an immune response.

25 In one aspect, the present invention provides FMDV polypeptides from ovine, bovine, caprine, or porcine. In another aspect, the present invention provides a polypeptide having a sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29, and variant or fragment thereof.

Moreover, homologs of FMDV polypeptides from ovine, bovine, caprine, or porcine  
30 are intended to be within the scope of the present invention. As used herein, the term “homologs” includes orthologs, analogs and paralogs. The term “analogs” refers to two polynucleotides or polypeptides that have the same or similar function, but that have evolved separately in unrelated organisms. The term “orthologs” refers to two polynucleotides or

polypeptides from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. The term "paralogs" refers to two polynucleotides or polypeptides that are related by duplication within a genome. Paralogs usually have different functions, but these functions  
5 may be related. Analogs, orthologs, and paralogs of a wild-type FMDV polypeptide can differ from the wild-type FMDV polypeptide by post-translational modifications, by amino acid sequence differences, or by both. In particular, homologs of the invention will generally exhibit at least 80-85%, 85-90%, 90-95%, or 95%, 96%, 97%, 98% , 99% sequence identity, with all or part of the wild-type FMDV or polynucleotide sequences, and will exhibit a  
10 similar function. Variants include allelic variants. The term "allelic variant" refers to a polynucleotide or a polypeptide containing polymorphisms that lead to changes in the amino acid sequences of a protein and that exist within a natural population (e.g., a virus species or variety). Such natural allelic variations can typically result in 1- 5% variance in a polynucleotide or a polypeptide. Allelic variants can be identified by sequencing the nucleic  
15 acid sequence of interest in a number of different species, which can be readily carried out by using hybridization probes to identify the same gene genetic locus in those species. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity of gene of interest, are intended to be within the scope of the invention.

20 As used herein, the term "derivative" or "variant" refers to a polypeptide, or a nucleic acid encoding a polypeptide, that has one or more conservative amino acid variations or other minor modifications such that (1) the corresponding polypeptide has substantially equivalent function when compared to the wild type polypeptide or (2) an antibody raised against the polypeptide is immunoreactive with the wild-type polypeptide. These variants or derivatives  
25 include polypeptides having minor modifications of the FMDV polypeptide primary amino acid sequences that may result in peptides which have substantially equivalent activity as compared to the unmodified counterpart polypeptide. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. The term "variant" further contemplates deletions, additions and substitutions to the sequence, so long as the  
30 polypeptide functions to produce an immunological response as defined herein.

The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically



similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, as described above.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code, e.g., optimized codon usage for a specific host. As used herein, “optimized” refers to a polynucleotide that is genetically engineered to increase its expression in a given species. To provide optimized polynucleotides coding for FMDV polypeptides, the DNA sequence of the FMDV protein gene can be modified to 1) comprise codons preferred by highly expressed genes in a particular species; 2) comprise an A+T or G+C content in nucleotide base composition to that substantially found in said species; 3) form an initiation sequence of said species; or 4) eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation and termination of RNA, or that form secondary structure hairpins or RNA splice sites. Increased expression of FMDV protein in said species can be achieved by utilizing the distribution frequency of codon usage in eukaryotes and prokaryotes, or in a particular species. The term “frequency of preferred codon usage” refers to the preference exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the FMDV polypeptide encoded by the nucleotide sequence is functionally unchanged.

The sequence identity between two amino acid sequences may be established by the NCBI (National Center for Biotechnology Information) pairwise blast and the blosum62 matrix, using the standard parameters (see, e.g., the BLAST or BLASTX algorithm available on the "National Center for Biotechnology Information" (NCBI, Bethesda, Md., USA) server, as well as in Altschul *et al.*; and thus, this document speaks of using the algorithm or the BLAST or BLASTX and BLOSUM62 matrix by the term “blasts”).

The “identity” with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics™ Suite, Intelligenetics Inc. CA). When RNA sequences are said to be similar,

or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Thus, RNA sequences are within the scope of the invention and can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

5           The sequence identity or sequence similarity of two amino acid sequences, or the sequence identity between two nucleotide sequences can be determined using Vector NTI software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA).

          The following documents provide algorithms for comparing the relative identity or homology of sequences, and additionally or alternatively with respect to the foregoing, the  
10   teachings in these references can be used for determining percent homology or identity: Needleman SB and Wunsch CD; Smith TF and Waterman MS; Smith TF, Waterman MS and Sadler JR; Feng DF and Dolittle RF; Higgins DG and Sharp PM; Thompson JD, Higgins DG and Gibson TJ; and, Devereux J, Haeberlie P and Smithies O. And, without undue experimentation, the skilled artisan can consult with many other programs or references for  
15   determining percent homology.

          Hybridization reactions can be performed under conditions of different “stringency.” Conditions that increase stringency of a hybridization reaction are well known. See for example, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook et al., 1989).

20           The invention further encompasses the FMDV polynucleotides contained in a vector molecule or an expression vector and operably linked to a promoter element and optionally to an enhancer.

          A “vector” refers to a recombinant DNA or RNA plasmid or virus that comprises a heterologous polynucleotide to be delivered to a target cell, either *in vitro* or *in vivo*. The  
25   heterologous polynucleotide may comprise a sequence of interest for purposes of prevention or therapy, and may optionally be in the form of an expression cassette. As used herein, a vector needs not be capable of replication in the ultimate target cell or subject. The term includes cloning vectors and viral vectors.

          The term “recombinant” means a polynucleotide semisynthetic, or synthetic origin  
30   which either does not occur in nature or is linked to another polynucleotide in an arrangement not found in nature.

          “Heterologous” means derived from a genetically distinct entity from the rest of the entity to which it is being compared. For example, a polynucleotide may be placed by genetic

engineering techniques into a plasmid or vector derived from a different source, and is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence other than the native sequence is a heterologous promoter.

5           The present invention relates to ovine, bovine, caprine and porcine vaccines or pharmaceutical or immunological compositions which may comprise an effective amount of a recombinant FMDV antigens and a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle.

10           The subject matter described herein is directed in part, to compositions and methods related to the FMDV antigen prepared in a plant or alga expression system that was highly immunogenic and protected animals against challenge from homologous and heterologous FMDV strains.

#### Compositions

15           The present invention relates to an FMDV vaccine or composition which may comprise an effective amount of a recombinant FMDV antigen and a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle. In one embodiment, the recombinant FMDV antigen is expressed in a plant or alga.

20           In an embodiment, the subject matter disclosed herein is directed to a composition comprising an FMDV antigen produced by a duckweed expression system and plant material from duckweed, including the genus *Lemna*, and a pharmaceutical or veterinarily acceptable carrier, excipient or vehicle. In another embodiment, the subject matter disclosed herein is directed to an optionally aglycosylated protein produced by a duckweed expression system comprising an FMDV antigen.

25           In one embodiment, the recombinant FMDV antigen is expressed in algae. In yet another embodiment, the algae are selected from *Schizochytrium*. In one embodiment, the recombinant FMDV antigen may be expressed in a *Schizochytrium* protein expression system, as described, for example, in US 7,001,772, US 2008/0022422, US provisional App. No. 61/160,618, and US provisional applications concurrently filed on December 28, 2009 by  
30   Martek BioScience Corp. (MD, USA).

          In an embodiment, the subject matter disclosed herein is directed to a protein produced by a plant or alga expression system comprising an FMDV antigen and material from the plant or alga.

In an embodiment, the subject matter disclosed herein is directed to a vaccine or composition comprising an FMDV antigen produced by a duckweed expression system.

In an embodiment, the subject matter disclosed herein is directed to a vaccine or composition comprising an FMDV antigen produced by a duckweed expression system and  
5 plant material from duckweed.

In an embodiment, the subject matter disclosed herein is directed to a stably transformed plant or plant culture that expresses an FMDV antigen wherein the plant or plant culture is selected from duckweed.

The present invention encompasses any FMDV polypeptide, antigen, epitope or  
10 immunogen that elicits an immunogenic response in an animal, such as an ovine, bovine, caprine or porcine. The FMDV polypeptide, antigen, epitope or immunogen may be any FMDV polypeptide, antigen, epitope or immunogen, such as, but not limited to, a protein, peptide or fragment thereof, that elicits, induces or stimulates a response in an animal, such as an ovine, bovine, caprine or porcine.

In an embodiment wherein the FMDV immunological composition or vaccine is a  
15 recombinant immunological composition or vaccine, the composition or vaccine comprising a recombinant vector and a pharmaceutical or veterinary acceptable excipient, carrier or vehicle; the recombinant vector is plant expression vector which may comprise a polynucleotide encoding a polypeptide, antigen, epitope or immunogen. The FMDV  
20 polypeptide, antigen, epitope or immunogen, may be VP1, VP2, VP3, VP4, VP5, NS1, VP7, NS2, VP6, NS3, NS3a, P1, VP0, 3C, or any fragment thereof.

In another embodiment, the FMDV antigen is P1, VP0, VP3, VP1, VP2, VP4, 2A, 2B, or 3C.

In an embodiment wherein the FMDV immunological composition or vaccine is a  
25 recombinant immunological composition or vaccine, the composition or vaccine comprising a recombinant vector and a pharmaceutical or veterinary acceptable excipient, carrier or vehicle; the recombinant vector is plant expression vector which may comprise a polynucleotide encoding an FMDV polypeptide, antigen, epitope or immunogen. The FMDV polypeptide, antigen, epitope or immunogen, may be an FMDV polypeptide VP1, VP2, VP3,  
30 VP4, 2A, 2B or 3C. In one embodiment, the nucleic acid molecule encoding one or more foot-and-mouth disease virus (FMDV) antigen(s) is a cDNA encoding FMDV P1 region and a cDNA encoding FMDV 3C protease of FMDV.

In one embodiment, the FMDV antigen may be a P1-3C polypeptide. In another embodiment, the FMDV antigen may be P1 alone, or P1-2A/2B1. In yet another embodiment, the FMDV antigen may be VP0-VP3. In another embodiment, the FMDV antigen may be VP4-VP2. In still another embodiment, the FMDV antigen may be 3C, or  
5 may be 3C with a 5'UTR optimized for expression in duckweed. In one embodiment, both P1-2A/2B1 and 3C polypeptides may be expressed in duckweed using a single construct and the expression may be regulated by one or more than one promoter sequences.

In another embodiment, the FMDV antigen may be *FMDV O1 Manisa, O1 BFS or Campos, A24 Cruzeiro, Asia 1 Shamir, A Iran '96, A22 Iraq, SAT2 Saudi Arabia.*

10 The present invention relates to an FMDV vaccine which may comprise an effective amount of a recombinant FMDV antigen and a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle. In one embodiment, the FMDV antigen may be FMDV P1, VP0, VP3, VP1, VP2, VP4, or 3C.

In another embodiment, the recombinant FMDV antigen is expressed in a plant or  
15 alga. In yet another embodiment, the plant is a duckweed plant, including a *Lemna* plant. In yet another embodiment, the plant is *Lemna minor*. In one embodiment, the recombinant FMDV antigen may be expressed in a proprietary *Lemna minor* protein expression system, advantageously Biolex's LEX system<sup>SM</sup>. In another embodiment, the algae are selected from *Schizochytrium*. In one embodiment, the recombinant FMDV antigen may be expressed in a  
20 *Schizochytrium* protein expression system, as described, for example, in US 7,001,772, US 2008/0022422, US 2010/0233760 A1 by Martek BioScience Corp. (MD, USA).

In another embodiment, pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle may be a water-in-oil emulsion. In yet another embodiment, the water-in-oil emulsion may be an oil-in-water emulsion.

25 The invention further encompasses the FMDV polynucleotides contained in a vector molecule or an expression vector and operably linked to a promoter element and optionally to an enhancer.

In one aspect, the present invention provides FMDV polypeptides, particularly ovine, bovine, caprine or porcine polypeptides having a sequence as set forth in SEQ ID NO:3 and  
30 variants or fragments thereof.

In another aspect, the present invention provides a polypeptide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99%

sequence identity to an antigenic polypeptide of the invention, particularly to the polypeptides having a sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29.

In yet another aspect, the present invention provides fragments and variants of the FMDV polypeptides identified above (SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29) which  
5 may readily be prepared by one of skill in the art using well-known molecular biology techniques.

Variants are homologous polypeptides having an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29.

10 An immunogenic fragment of an FMDV polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids, at least 21 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids of an FMDV polypeptide having a sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29, or variants thereof. In another embodiment, a fragment of an FMDV polypeptide includes a specific antigenic epitope found on a full-  
15 length FMDV polypeptide.

In another aspect, the present invention provides a polynucleotide encoding an FMDV polypeptide, such as a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29. In yet another aspect, the present invention provides a polynucleotide encoding a polypeptide having at least 70%, at least 75%, at least  
20 80%, at least 85%, at least 90%, at least 95%, 96%, 97%, 98% or 99% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29, or a conservative variant, an allelic variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides.

25 In another aspect, the present invention provides a polynucleotide having a nucleotide sequence as set forth in SEQ ID NOs:1, 2, 4, 8, 9, 11, 13, 14, 15, 16, 18, 19, 21, 22, 24, 25, 27, 28, 30-35, or a variant thereof. In yet another aspect, the present invention provides a polynucleotide having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 95%, 96%, 97%, 98% or 99% sequence identity to one of a polynucleotide  
30 having a sequence as set forth in SEQ ID NOs:1, 2, 4, 8, 9, 11, 13, 14, 15, 16, 18, 19, 21, 22, 24, 25, 27, 28, 30-35, or a variant thereof.

The polynucleotides of the invention may comprise additional sequences, such as additional encoding sequences within the same transcription unit, controlling elements such

as promoters, ribosome binding sites, 5'UTR, 3'UTR, transcription terminators, polyadenylation sites, additional transcription units under control of the same or a different promoter, sequences that permit cloning, expression, homologous recombination, and transformation of a host cell, and any such construct as may be desirable to provide  
5 embodiments of this invention.

Elements for the expression of an FMDV polypeptide, antigen, epitope or immunogen are advantageously present in an inventive vector. In minimum manner, this comprises, consists essentially of, or consists of an initiation codon (ATG), a stop codon and a promoter, and optionally also a polyadenylation sequence for certain vectors such as plasmid and  
10 certain viral vectors, e.g., viral vectors other than poxviruses. When the polynucleotide encodes a polyprotein fragment, e.g. an FMDV peptide, advantageously, in the vector, an ATG is placed at 5' of the reading frame and a stop codon is placed at 3'. Other elements for controlling expression may be present, such as enhancer sequences, stabilizing sequences, such as intron and signal sequences permitting the secretion of the protein.

The present invention also relates to preparations comprising vectors, such as expression vectors, e.g., therapeutic compositions. The preparations can comprise one or more vectors, e.g., expression vectors, such as *in vivo* expression vectors, comprising and expressing one or more FMDV polypeptides, antigens, epitopes or immunogens. In one embodiment, the vector contains and expresses a polynucleotide that comprises, consists  
20 essentially of, or consists of a polynucleotide coding for (and advantageously expressing) an FMDV antigen, epitope or immunogen, in a pharmaceutically or veterinarily acceptable carrier, excipient or vehicle. Thus, according to an embodiment of the invention, the other vector or vectors in the preparation comprises, consists essentially of or consists of a polynucleotide that encodes, and under appropriate circumstances the vector expresses one or  
25 more other proteins of an FMDV polypeptide, antigen, epitope or immunogen, or a fragment thereof.

According to another embodiment, the vector or vectors in the preparation comprise, or consist essentially of, or consist of polynucleotide(s) encoding one or more proteins or fragment(s) thereof of an FMDV polypeptide, antigen, epitope or immunogen, the vector or  
30 vectors expressing the polynucleotide(s). In another embodiment, the preparation comprises one, two, or more vectors comprising polynucleotides encoding and expressing, advantageously *in vivo*, an FMDV polypeptide, antigen, fusion protein or an epitope thereof. The invention is also directed at mixtures of vectors that comprise polynucleotides encoding

and expressing different FMDV polypeptides, antigens, epitopes or immunogens, e.g., an FMDV polypeptide, antigen, epitope or immunogen from different animal species such as, but not limited to, ovine, bovine, caprine or porcine

According to a yet further embodiment of the invention, the expression vector is a plasmid vector or a DNA plasmid vector, in particular an *in vivo* expression vector. In a specific, non-limiting example, the pVR1020 or 1012 plasmid (VICAL Inc.; Luke et al., 1997; Hartikka et al., 1996, see, e.g., U.S. Patent Nos. 5,846,946 and 6,451,769) can be utilized as a vector for the insertion of a polynucleotide sequence. The pVR1020 plasmid is derived from pVR1012 and contains the human tPA signal sequence. In one embodiment the human tPA signal comprises from amino acid M(1) to amino acid S(23) in Genbank under the accession number HUMTPA14. In another specific, non-limiting example, the plasmid utilized as a vector for the insertion of a polynucleotide sequence can contain the signal peptide sequence of equine IGF1 from amino acid M(24) to amino acid A(48) in Genbank under the accession number U28070. Additional information on DNA plasmids which may be consulted or employed in the practice are found, for example, in U.S. Patent Nos. 6,852,705; 6,818,628; 6,586,412; 6,576,243; 6,558,674; 6,464,984; 6,451,770; 6,376,473 and 6,221,362.

The term plasmid covers any DNA transcription unit comprising a polynucleotide according to the invention and the elements necessary for its *in vivo* expression in a cell or cells of the desired host or target; and, in this regard, it is noted that a supercoiled or non-supercoiled, circular plasmid, as well as a linear form, are intended to be within the scope of the invention.

Each plasmid comprises or contains or consists essentially of, in addition to the polynucleotide encoding an FMDV antigen, epitope or immunogen, optionally fused with a heterologous peptide sequence, variant, analog or fragment, operably linked to a promoter or under the control of a promoter or dependent upon a promoter. In general, it is advantageous to employ a strong promoter functional in eukaryotic cells. The strong promoter may be, but not limited to, the immediate early cytomegalovirus promoter (CMV-IE) of human or murine origin, or optionally having another origin such as the rat or guinea pig, the Super promoter (Ni, M. et al., Plant J. 7, 661-676, 1995.). The CMV-IE promoter can comprise the actual promoter part, which may or may not be associated with the enhancer part. Reference can be made to EP-A-260 148, EP-A-323 597, U.S. Patents Nos. 5,168,062, 5,385,839, and



4,968,615, as well as to PCT Application No WO87/03905. The CMV-IE promoter is advantageously a human CMV-IE (Boshart et al., 1985) or murine CMV-IE.

In more general terms, the promoter has either a viral, a plant, or a cellular origin. A strong viral promoter other than CMV-IE that may be usefully employed in the practice of the invention is the early/late promoter of the SV40 virus or the LTR promoter of the Rous sarcoma virus. A strong cellular promoter that may be usefully employed in the practice of the invention is the promoter of a gene of the cytoskeleton, such as e.g. the desmin promoter (Kwissa et al., 2000), or the actin promoter (Miyazaki et al., 1989).

Any of constitutive, regulatable, or stimulus-dependent promoters may be used. For example, constitutive promoters may include the mannopine synthase promoter from *Agrobacterium tumefaciens*. Alternatively, it may be advantageous to use heat shock gene promoters, drought-inducible gene promoters, pathogen-inducible gene promoters, wound-inducible gene promoters, and light/dark-inducible gene promoters. It may be useful to use promoters that are controlled by plant growth regulators, such as abscissic acid, auxins, cytokinins, and gibberellic acid. Promoters may also be chosen that give tissue-specific expression (e.g., root, leaf, and floral-specific promoters).

The plasmids may comprise other expression control elements. It is particularly advantageous to incorporate stabilizing sequence(s), e.g., intron sequence(s), for example, maize alcohol dehydrogenase intron (Callis et al. *Genes & Dev.*1(10):1183-1200, Dec. 1987), the first intron of the hCMV-IE (PCT Application No. WO1989/01036), the intron II of the rabbit  $\beta$ -globin gene (van Ooyen et al., 1979). In another embodiment, the plasmids may comprise 3' UTR. The 3' UTR may be, but not limited to, *agrobacterium* nopaline synthase (Nos) 3' UTR (Nopaline synthase: transcript mapping and DNA sequence. Depicker, A. et al. *J. Mol. Appl. Genet.*, 1982; Bevan, NAR, 1984, 12(22): 8711-8721).

As to the polyadenylation signal (polyA) for the plasmids and viral vectors other than poxviruses, use can more be made of the poly(A) signal of the bovine growth hormone (bGH) gene (see U.S. 5,122,458), or the poly(A) signal of the rabbit  $\beta$ -globin gene or the poly(A) signal of the SV40 virus.

A "host cell" denotes a prokaryotic or eukaryotic cell that has been genetically altered, or is capable of being genetically altered by administration of an exogenous polynucleotide, such as a recombinant plasmid or vector. When referring to genetically altered cells, the term refers both to the originally altered cell and to the progeny thereof.

In one embodiment, the recombinant FMDV antigen is expressed in a transgenic plant or alga. In another embodiment, the transgenic plant is a *Lemna* plant. In yet another embodiment, the transgenic plant is *Lemna minor* (duckweed). In yet another embodiment, the recombinant FMDV antigen may be expressed in the *Lemna minor* (duckweed) protein expression system, the Biolex's LEX system<sup>SM</sup>. Details of the *Lemna minor* (duckweed) protein expression system may be found, for example, in U.S. Patent Nos. 6,815,184, 7,022,309, 7,160,717, 7,176,024, 6,040,498, and 7,161,064, the disclosures of which are incorporated by reference in their entireties. In yet another embodiment, the transgenic alga is *Schizochytrium*. Details of the algal protein expression system may be found, for example, in US 7,001,772, US 2008/0022422, US provisional application 61/160,618, the disclosures of which are incorporated by reference in their entireties. The FMDV antigen in the embodiments may be any polypeptide disclosed herein, or a polypeptide encoded by any polynucleotide disclosed herein.

#### 15 Methods for Expressing FMDV polypeptides in Duckweed or Microalga

Thus, in some embodiments of the invention, antigenic FMDV polypeptides, or fragments or variants thereof, are expressed in duckweed or microalga. These methods comprise the use of expression cassettes that are introduced into a duckweed plant or microalga using any suitable transformation method known in the art. Polynucleotides within these expression cassettes can be modified for enhanced expression of the antigenic FMDV polypeptide, or fragment or variant thereof, in duckweed or microalga, as follows.

##### Cassettes for Duckweed or Microalga Expression of Antigenic FMDV Polypeptides

Transgenic duckweed or microalga expressing an antigenic FMDV polypeptide, or fragment or variant thereof, is obtained by transformation of duckweed or microalga with an expression cassette comprising a polynucleotide encoding the antigenic FMDV polypeptide, or fragment or variant thereof. In this manner, a polynucleotide encoding the antigenic FMDV polypeptide of interest, or fragment or variant thereof, is constructed within an expression cassette and introduced into a duckweed plant or microalga culture by any suitable transformation method known in the art.

In some embodiments, the duckweed plant or microalga that is transformed with an expression cassette comprising polynucleotide encoding the antigenic FMDV polypeptide of interest, or fragment or variant thereof, has also been transformed with an expression cassette that provides for expression of another heterologous polypeptide of interest, for example,

another antigenic FMDV polypeptide, fragment, or variant thereof. The expression cassette providing for expression of another heterologous polypeptide of interest can be provided on the same polynucleotide (for example, on the same transformation vector) for introduction into a duckweed plant or microalga, or on a different polynucleotide (for example, on  
5 different transformation vectors) for introduction into the duckweed plant or microalga at the same time or at different times, by the same or by different methods of introduction, for example, by the same or different transformation methods.

The expression cassettes for use in transformation of duckweed or microalga comprise expression control elements that at least comprise a transcriptional initiation region  
10 (e.g., a promoter) operably linked to the polynucleotide of interest, i.e., a polynucleotide encoding an antigenic FMDV polypeptide, fragment, or variant thereof. "Operably linked" as used herein in reference to nucleotide sequences refers to multiple nucleotide sequences that are placed in a functional relationship with each other. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in reading  
15 frame. Such an expression cassette is provided with a plurality of restriction sites for insertion of the polynucleotide or polynucleotides of interest (e.g., one polynucleotide of interest, two polynucleotides of interest, etc.) to be under the transcriptional regulation of the promoter and other expression control elements. In particular embodiments of the invention, the polynucleotide to be transferred contains two or more expression cassettes, each of which  
20 contains at least one polynucleotide of interest.

By "expression control element" is intended a regulatory region of DNA, usually comprising a TATA box, capable of directing RNA polymerase II, or in some embodiments, RNA polymerase III, to initiate RNA synthesis at the appropriate transcription initiation site for a particular coding sequence. An expression control element may additionally comprise  
25 other recognition sequences generally positioned upstream or 5' to the TATA box, which influence (e.g., enhance) the transcription initiation rate. Furthermore, an expression control element may additionally comprise sequences generally positioned downstream or 3' to the TATA box, which influence (e.g., enhance) the transcription initiation rate.

The transcriptional initiation region (e.g., a promoter) may be native or homologous  
30 or foreign or heterologous to the duckweed or microalga host, or could be the natural sequence or a synthetic sequence. By foreign, it is intended that the transcriptional initiation region is not found in the wild-type duckweed or microalga host into which the transcriptional initiation region is introduced. By "functional promoter" is intended the

promoter, when operably linked to a sequence encoding an antigenic FMDV polypeptide of interest, or fragment or variant thereof, is capable of driving expression (i.e., transcription and translation) of the encoded polypeptide, fragment, or variant. The promoters can be selected based on the desired outcome. Thus the expression cassettes of the invention can comprise  
5 constitutive, inducible, tissue-preferred, or other promoters for expression in duckweed.

Any suitable promoter known in the art can be employed in the expression cassettes according to the present invention, including bacterial, yeast, fungal, insect, mammalian, and plant promoters. For example, plant promoters, including duckweed or microalga promoters, may be used. Exemplary promoters include, but are not limited to, the Cauliflower Mosaic  
10 Virus 35S promoter, the opine synthetase promoters (e.g., nos, mas, ocs, etc.), the ubiquitin promoter, the actin promoter, the ribulose biphosphate (RubP) carboxylase small subunit promoter, and the alcohol dehydrogenase promoter. The duckweed RubP carboxylase small subunit promoter is known in the art (Silverthorne *et al.* (1990) *Plant Mol. Biol.* 15:49). Other promoters from viruses that infect plants or microalgae are also suitable, including, but not  
15 limited to, promoters isolated from Dasheen mosaic virus, Chlorella virus (e.g., the Chlorella virus adenine methyltransferase promoter; Mitra *et al.* (1994) *Plant Mol. Biol.* 26:85), tomato spotted wilt virus, tobacco rattle virus, tobacco necrosis virus, tobacco ring spot virus, tomato ring spot virus, cucumber mosaic virus, peanut stump virus, alfalfa mosaic virus, sugarcane bacilliform badnavirus and the like.

Expression control elements, including promoters, can be chosen to give a desired  
20 level of regulation. For example, in some instances, it may be advantageous to use a promoter that confers constitutive expression (e.g., the mannopine synthase promoter from *Agrobacterium tumefaciens*). Alternatively, in other situations, it may be advantageous to use promoters that are activated in response to specific environmental stimuli (e.g., heat shock  
25 gene promoters, drought-inducible gene promoters, pathogen-inducible gene promoters, wound-inducible gene promoters, and light/dark-inducible gene promoters) or plant growth regulators (e.g., promoters from genes induced by abscissic acid, auxins, cytokinins, and gibberellic acid). As a further alternative, promoters can be chosen that give tissue-specific expression (e.g., root, leaf, and floral-specific promoters).

The overall strength of a given promoter can be influenced by the combination and  
30 spatial organization of cis-acting nucleotide sequences such as upstream activating sequences. For example, activating nucleotide sequences derived from the *Agrobacterium tumefaciens* octopine synthase gene can enhance transcription from the *Agrobacterium*

*tumefaciens* mannopine synthase promoter (see U.S. Patent 5,955,646 to Gelvin *et al.*). In the present invention, the expression cassette can contain activating nucleotide sequences inserted upstream of the promoter sequence to enhance the expression of the antigenic FMDV polypeptide of interest, or fragment or variant thereof. In one embodiment, the expression cassette includes three upstream activating sequences derived from the *Agrobacterium tumefaciens* octopine synthase gene operably linked to a promoter derived from an *Agrobacterium tumefaciens* mannopine synthase gene (see U.S. Patent 5,955,646, herein incorporated by reference).

The expression cassette thus includes in the 5'-3' direction of transcription, an expression control element comprising a transcriptional and translational initiation region, a polynucleotide of encoding an antigenic FMDV polypeptide of interest (or fragment or variant thereof), and a transcriptional and translational termination region functional in plants. Any suitable termination sequence known in the art may be used in accordance with the present invention. The termination region may be native with the transcriptional initiation region, may be native with the coding sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthetase and nopaline synthetase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141; Proudfoot (1991) *Cell* 64:671; Sanfacon *et al.* (1991) *Genes Dev.* 5:141; Mogen *et al.* (1990) *Plant Cell* 2:1261; Munroe *et al.* (1990) *Gene* 91:151; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891; and Joshi *et al.* (1987) *Nucleic Acids Res.* 15:9627. Additional exemplary termination sequences are the pea RubP carboxylase small subunit termination sequence and the Cauliflower Mosaic Virus 35S termination sequence.

Generally, the expression cassette will comprise a selectable marker gene for the selection of transformed duckweed cells or tissues. Selectable marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds. Herbicide resistance genes generally code for a modified target protein insensitive to the herbicide or for an enzyme that degrades or detoxifies the herbicide in the plant before it can act. See DeBlock *et al.* (1987) *EMBO J.* 6:2513; DeBlock *et al.* (1989) *Plant Physiol.* 91:691; Fromm *et al.* (1990) *BioTechnology* 8:833; Gordon-Kamm *et al.* (1990) *Plant Cell* 2:603. For example, resistance to glyphosate or sulfonylurea herbicides has been obtained using genes coding for the mutant target enzymes, 5-

enolpyruvylshikimate-3-phosphate synthase (EPSPS) and acetolactate synthase (ALS). Resistance to glufosinate ammonium, boromoxynil, and 2,4-dichlorophenoxyacetate (2,4-D) have been obtained by using bacterial genes encoding phosphinothricin acetyltransferase, a nitrilase, or a 2,4-dichlorophenoxyacetate monooxygenase, which detoxify the respective  
5 herbicides.

For purposes of the present invention, selectable marker genes include, but are not limited to, genes encoding neomycin phosphotransferase II (Fraley *et al.* (1986) *CRC Critical Reviews in Plant Science* 4:1); cyanamide hydratase (Maier-Greiner *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:4250); aspartate kinase; dihydrodipicolinate synthase (Perl *et al.* (1993)  
10 *BioTechnology* 11:715); bar gene (Toki *et al.* (1992) *Plant Physiol.* 100:1503; Meagher *et al.* (1996) *Crop Sci.* 36:1367); tryptophan decarboxylase (Goddijn *et al.* (1993) *Plant Mol. Biol.* 22:907); neomycin phosphotransferase (NEO; Southern *et al.* (1982) *J. Mol. Appl. Gen.* 1:327); hygromycin phosphotransferase (HPT or HYG; Shimizu *et al.* (1986) *Mol. Cell. Biol.* 6:1074); dihydrofolate reductase (DHFR; Kwok *et al.* (1986) *Proc. Natl. Acad. Sci. USA*  
15 83:4552); phosphinothricin acetyltransferase (DeBlock *et al.* (1987) *EMBO J.* 6:2513); 2,2-dichloropropionic acid dehalogenase (Buchanan-Wollatton *et al.* (1989) *J. Cell. Biochem.* 13D:330); acetohydroxyacid synthase (U.S. Pat. No. 4,761,373 to Anderson *et al.*; Haughn *et al.* (1988) *Mol. Gen. Genet.* 221:266); 5-enolpyruvyl-shikimate-phosphate synthase (aroA; Comai *et al.* (1985) *Nature* 317:741); haloarylnitrilase (WO 87/04181 to Stalker *et al.*);  
20 acetyl-coenzyme A carboxylase (Parker *et al.* (1990) *Plant Physiol.* 92:1220); dihydropteroate synthase (sulI; Guerineau *et al.* (1990) *Plant Mol. Biol.* 15:127); and 32 kDa photosystem II polypeptide (psbA; Hirschberg *et al.* (1983) *Science* 222:1346 (1983).

Also included are genes encoding resistance to: gentamycin (*e.g.*, aacC1, Wohlleben *et al.* (1989) *Mol. Gen. Genet.* 217:202-208); chloramphenicol (Herrera-Estrella *et al.* (1983)  
25 *EMBO J.* 2:987); methotrexate (Herrera-Estrella *et al.* (1983) *Nature* 303:209; Meijer *et al.* (1991) *Plant Mol. Biol.* 16:807); hygromycin (Waldron *et al.* (1985) *Plant Mol. Biol.* 5:103; Zhijian *et al.* (1995) *Plant Science* 108:219; Meijer *et al.* (1991) *Plant Mol. Bio.* 16:807); streptomycin (Jones *et al.* (1987) *Mol. Gen. Genet.* 210:86); spectinomycin (Bretagne-Sagnard *et al.* (1996) *Transgenic Res.* 5:131); bleomycin (Hille *et al.* (1986) *Plant Mol. Biol.*  
30 7:171); sulfonamide (Guerineau *et al.* (1990) *Plant Mol. Bio.* 15:127); bromoxynil (Stalker *et al.* (1988) *Science* 242:419); 2,4-D (Streber *et al.* (1989) *BioTechnology* 7:811); phosphinothricin (DeBlock *et al.* (1987) *EMBO J.* 6:2513); spectinomycin (Bretagne-Sagnard and Chupeau, *Transgenic Research* 5:131).

The bar gene confers herbicide resistance to glufosinate-type herbicides, such as phosphinothricin (PPT) or bialaphos, and the like. As noted above, other selectable markers that could be used in the vector constructs include, but are not limited to, the pat gene, also for bialaphos and phosphinothricin resistance, the ALS gene for imidazolinone resistance, the HPH or HYG gene for hygromycin resistance, the EPSP synthase gene for glyphosate resistance, the Hm1 gene for resistance to the Hc-toxin, and other selective agents used routinely and known to one of ordinary skill in the art. See Yarranton (1992) *Curr. Opin. Biotech.* 3:506; Christopherson *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6314; Yao *et al.* (1992) *Cell* 71:63; Reznikoff (1992) *Mol. Microbiol.* 6:2419; Barkley *et al.* (1980) *The Operon* 177-220; Hu *et al.* (1987) *Cell* 48:555; Brown *et al.* (1987) *Cell* 49:603; Figge *et al.* (1988) *Cell* 52:713; Deuschle *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:5400; Fuerst *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2549; Deuschle *et al.* (1990) *Science* 248:480; Labow *et al.* (1990) *Mol. Cell. Biol.* 10:3343; Zambretti *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3952; Baim *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:5072; Wyborski *et al.* (1991) *Nuc. Acids Res.* 19:4647; Hillenand-Wissman (1989) *Topics in Mol. And Struc. Biol.* 10:143; Degenkolb *et al.* (1991) *Antimicrob. Agents Chemother.* 35:1591; Kleinschmidt *et al.* (1988) *Biochemistry* 27:1094; Gatz *et al.* (1992) *Plant J.* 2:397; Gossen *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:5547; Oliva *et al.* (1992) *Antimicrob. Agents Chemother.* 36:913; Hlavka *et al.* (1985) *Handbook of Experimental Pharmacology* 78; and Gill *et al.* (1988) *Nature* 334:721. Such disclosures are herein incorporated by reference.

The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

#### Modification of Nucleotide Sequences for Enhanced Expression in a Plant or microalga Host

Where the antigenic FMDV polypeptide or fragment or variant thereof is expressed within duckweed or microalga, the expressed polynucleotide sequence encoding the FMDV polypeptide or fragment or variant thereof can be modified to enhance its expression in duckweed or microalga, respectively. One such modification is the synthesis of the polynucleotide using plant-preferred codons, particularly duckweed-preferred codons, or using microalga-preferred codons, such as *Schizochytrium*-preferred codons. Methods are available in the art for synthesizing nucleotide sequences with plant-preferred codons. See, e.g., U.S. Patent Nos. 5,380,831 and 5,436,391; EP 0 359 472; EP 0 385 962; WO 91/16432;

Perlak *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 15:3324; Iannacome *et al.* (1997) *Plant Mol. Biol.* 34:485; and Murray *et al.* (1989) *Nucleic Acids. Res.* 17:477, herein incorporated by reference. Synthesis can be accomplished using any method known to one of skill in the art. The preferred codons may be determined from the codons of highest frequency in the proteins expressed in duckweed or microalga. For example, the frequency of codon usage for *Lemna minor* is found in Table 1, the frequency of codon usage for *Schizochytrium* is found in Table 2.

Table 1. *Lemna minor* [gbpln]: 4 CDS's (1597 codons)

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fields: [triplet] [frequency: **per thousand**] ([number])

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15

UUU 17.5 (28)	UCU 13.8 (22)	UAU 8.8 (14)	UGU 5.0 (8)
UUC 36.3 (58)	UCC 17.5 (28)	UAC 15.7 (25)	UGC 14.4 (23)
UUA 5.6 (9)	UCA 14.4 (23)	UAA 0.0 (0)	UGA 1.9 (3)
UUG 13.8 (22)	UCG 13.8 (22)	UAG 0.6 (1)	UGG 16.3 (26)

20

CUU 15.7 (25)	CCU 11.9 (19)	CAU 6.9 (11)	CGU 4.4 (7)
CUC 25.7 (41)	CCC 15.7 (25)	CAC 16.9 (27)	CGC 18.2 (29)
CUA 5.0 (8)	CCA 11.3 (18)	CAA 10.0 (16)	CGA 6.3 (10)
CUG 21.3 (34)	CCG 14.4 (23)	CAG 22.5 (36)	CGG 10.6 (17)

25

AUU 18.8 (30)	ACU 9.4 (15)	AAU 13.8 (22)	AGU 10.0 (16)
AUC 19.4 (31)	ACC 17.5 (28)	AAC 21.9 (35)	AGC 15.0 (24)
AUA 1.9 (3)	ACA 5.0 (8)	AAA 15.7 (25)	AGA 20.7 (33)
AUG 20.7 (33)	ACG 10.0 (16)	AAG 35.7 (57)	AGG 17.5 (28)

30

GUU 15.0 (24)	GCU 25.0 (40)	GAU 20.0 (32)	GGU 8.1 (13)
GUC 25.0 (40)	GCC 22.5 (36)	GAC 26.3 (42)	GGC 21.9 (35)
GUA 6.3 (10)	GCA 14.4 (23)	GAA 26.3 (42)	GGA 16.9 (27)
GUG 30.7 (49)	GCG 18.2 (29)	GAG 40.1 (64)	GGG 18.2 (29)

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35



Table 2. *Schizochytrium sp. ATCC\_20888* [gbpln]: 3 CDS's (6473 codons)

fields: [triplet] [frequency: per thousand] ([number])

5	UUU 12.2 (79)	UCU 7.0 (45)	UAU 1.1 (7)	UGU 0.8 (5)
	UUC 19.9 (129)	UCC 23.8 (154)	UAC 21.5 (139)	UGC 15.3 (99)
	UUA 0.0 (0)	UCA 0.5 (3)	UAA 0.5 (3)	UGA 0.0 (0)
	UUG 0.6 (4)	UCG 18.8 (122)	UAG 0.0 (0)	UGG 8.3 (54)
10	CUU 12.7 (82)	CCU 11.7 (76)	CAU 2.3 (15)	CGU 7.1 (46)
	CUC 61.2 (396)	CCC 23.8 (154)	CAC 12.8 (83)	CGC 42.9 (278)
	CUA 0.0 (0)	CCA 1.5 (10)	CAA 2.3 (15)	CGA 0.3 (2)
	CUG 7.4 (48)	CCG 16.2 (105)	CAG 27.7 (179)	CGG 0.8 (5)
15	AUU 13.9 (90)	ACU 9.1 (59)	AAU 1.9 (12)	AGU 1.5 (10)
	AUC 33.5 (217)	ACC 29.2 (189)	AAC 32.4 (210)	AGC 15.6 (101)
	AUA 0.0 (0)	ACA 1.5 (10)	AAA 2.2 (14)	AGA 0.2 (1)
	AUG 27.8 (180)	ACG 9.6 (62)	AAG 54.5 (353)	AGG 0.0 (0)
20	GUU 8.3 (54)	GCU 24.4 (158)	GAU 13.4 (87)	GGU 13.0 (84)
	GUC 53.0 (343)	GCC 86.0 (557)	GAC 45.0 (291)	GGC 54.5 (353)
	GUA 0.2 (1)	GCA 4.0 (26)	GAA 7.3 (47)	GGA 3.9 (25)
	GUG 14.4 (93)	GCG 15.9 (103)	GAG 62.3 (403)	GGG 0.5 (3)

25 For purposes of the present invention, “duckweed-preferred codons” refers to codons that have a frequency of codon usage in duckweed of greater than 17%. “*Lemna*-preferred codons” as used herein refers to codons that have a frequency of codon usage in the genus *Lemna* of greater than 17%. “*Lemna minor*-preferred codons” as used herein refers to codons that have a frequency of codon usage in *Lemna minor* of greater than 17% where the  
 30 frequency of codon usage in *Lemna minor* is obtained from the Codon Usage Database (GenBank Release 160.0, June 15, 2007). “Microalgae-preferred codons” refers to codons that have a frequency of codon usage in microalgae of greater than 17%. “microalgae-preferred codons” as used herein refers to codons that have a frequency of codon usage in the family *Thraustochytriaceae* of greater than 17%. “*Schizochytrium*-preferred codons” as used  
 35 herein refers to codons that have a frequency of codon usage in *schizochytrium* of greater

than 17% where the frequency of codon usage in *schizochytrium* is obtained from the Codon Usage Database.

It is further recognized that all or any part of the polynucleotide encoding the antigenic FMDV polypeptide of interest, or fragment or variant thereof, may be optimized or synthetic. In other words, fully optimized or partially optimized sequences may also be used. For example, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the codons may be duckweed-preferred or microalgae-preferred codons. In one embodiment, between 90 and 96% of the codons are duckweed-preferred or microalgae-preferred codons. The coding sequence of a polynucleotide sequence encoding an antigenic FMDV polypeptide of interest, or fragment or variant thereof, may comprise codons used with a frequency of at least 17% in *Lemna gibba* or at least 17% in *Lemna minor*. In another such embodiment, the expression cassette comprises SEQ ID NO:9, which contains *Lemna minor*-preferred codons encoding the P1 polypeptide set forth in SEQ ID NO:10. In a related embodiment, the FMDV polypeptide is a P1-3C polypeptide, for example, the P1-3C polypeptide as set forth in SEQ ID NO:3, and the expression cassette comprises an optimized coding sequence for this P1-3C polypeptide, where the coding sequence comprises duckweed-preferred codons, for example, *Lemna minor*-preferred or *Lemna gibba*-preferred codons. In one such embodiment, the expression cassette comprises SEQ ID NO:2, which contains *Lemna minor*-preferred codons encoding the FMDV polypeptide as set forth in SEQ ID NO:3.

Other modifications can also be made to the polynucleotide encoding the antigenic FMDV polypeptide of interest, or fragment or variant thereof, to enhance its expression in duckweed or microalga. These modifications include, but are not limited to, elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for duckweed, as calculated by reference to known genes expressed in this plant. When possible, the polynucleotide encoding the heterologous polypeptide of interest may be modified to avoid predicted hairpin secondary mRNA structures.

There are known differences between the optimal translation initiation context nucleotide sequences for translation initiation codons in animals, plants and algae. "Translation initiation context nucleotide sequence" as used herein refers to the identity of the three nucleotides directly 5' of the translation initiation codon. "Translation initiation

codon" refers to the codon that initiates the translation of the mRNA transcribed from the nucleotide sequence of interest. The composition of these translation initiation context nucleotide sequences can influence the efficiency of translation initiation. See, for example, Lukaszewicz *et al.* (2000) *Plant Science* 154:89-98; and Joshi *et al.* (1997); *Plant Mol. Biol.* 5 35:993-1001. In the present invention, the translation initiation context nucleotide sequence for the translation initiation codon of the polynucleotide encoding the antigenic FMDV polypeptide of interest, or fragment or variant thereof, may be modified to enhance expression in duckweed. In one embodiment, the nucleotide sequence is modified such that the three nucleotides directly upstream of the translation initiation codon are "ACC." In a 10 second embodiment, these nucleotides are "ACA."

Expression of an antigenic FMDV polypeptide in duckweed or alga can also be enhanced by the use of 5' leader sequences. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include, but are not limited to, picornavirus leaders, e.g., EMCV leader (Encephalomyocarditis 5' noncoding region; Elroy-Stein *et al.* (1989) *Proc. Natl. Acad. Sci USA* 86:6126); potyvirus leaders, e.g., TEV leader (Tobacco Etch Virus; Allison *et al.* (1986) *Virology* 154:9); human immunoglobulin heavy-chain binding protein (BiP; Macajak and Sarnow (1991) *Nature* 353:90); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4; Jobling and Gehrke (1987) *Nature* 325:622); tobacco mosaic virus leader (TMV; Gallie (1989) *Molecular Biology of RNA*, 23:56); potato etch virus leader (Tomashevskaya *et al.* (1993) *J. Gen. Virol.* 20 74:2717-2724); Fed-1 5' untranslated region (Dickey (1992) *EMBO J.* 11:2311-2317); RbcS 5' untranslated region (Silverthorne *et al.* (1990) *J. Plant. Mol. Biol.* 15:49-58); and maize chlorotic mottle virus leader (MCMV; Lommel *et al.* (1991) *Virology* 81:382). See also, Della-Cioppa *et al.* (1987) *Plant Physiology* 84:965. Leader sequence comprising plant intron 25 sequence, including intron sequence from the maize alcohol dehydrogenase 1 (ADH1) gene, the castor bean catalase gene, or the *Arabidopsis* tryptophan pathway gene PAT1 has also been shown to increase translational efficiency in plants (Callis *et al.* (1987) *Genes Dev.* 1:1183-1200; Mascarenhas *et al.* (1990) *Plant Mol. Biol.* 15:913-920).

In some embodiments of the present invention, nucleotide sequence corresponding to 30 nucleotides 1222-1775 of the maize alcohol dehydrogenase 1 gene (SEQ ID NO:4; ADH1; GenBank Accession Number X04049) is inserted upstream of the polynucleotide encoding the antigenic FMDV polypeptide of interest, or fragment or variant thereof, to enhance the efficiency of its translation. In another embodiment, the expression cassette contains the

leader from the *Lemna gibba* ribulose-bis-phosphate carboxylase small subunit 5B gene (RbcS leader; see Buzby *et al.* (1990) *Plant Cell* 2:805-814).

It is recognized that any of the expression-enhancing nucleotide sequence modifications described above can be used in the present invention, including any single  
5 modification or any possible combination of modifications. The phrase “modified for enhanced expression” in duckweed, as used herein, refers to a polynucleotide sequence that contains any one or any combination of these modifications.

*Transformed Duckweed Plants and Duckweed Nodule Cultures or Transformed Microalgae*

10 The present invention provides transformed duckweed plants expressing an antigenic FMDV polypeptide of interest, or fragment or variant thereof. The term “duckweed” refers to members of the family *Lemnaceae*. This family currently is divided into five genera and 38 species of duckweed as follows: genus *Lemna* (*L. aequinoctialis*, *L. disperma*, *L. ecuadoriensis*, *L. gibba*, *L. japonica*, *L. minor*, *L. miniscula*, *L. obscura*, *L. perpusilla*, *L.*  
15 *tenera*, *L. trisulca*, *L. turionifera*, *L. valdiviana*); genus *Spirodela* (*S. intermedia*, *S. polyrrhiza*, *S. punctata*); genus *Wolffia* (*Wa. angusta*, *Wa. arrhiza*, *Wa. australina*, *Wa. borealis*, *Wa. brasiliensis*, *Wa. columbiana*, *Wa. elongata*, *Wa. globosa*, *Wa. microscopica*, *Wa. neglecta*); genus *Wolffiella* (*Wl. caudata*, *Wl. denticulata*, *Wl. gladiata*, *Wl. hyalina*, *Wl. lingulata*, *Wl. repunda*, *Wl. rotunda*, and *Wl. neotropica*) and genus *Landoltia* (*L. punctata*).  
20 Any other genera or species of *Lemnaceae*, if they exist, are also aspects of the present invention. *Lemna* species can be classified using the taxonomic scheme described by Landolt (1986) *Biosystematic Investigation on the Family of Duckweeds: The family of Lemnaceae—A Monograph Study* (Geobotanischen Institut ETH, Stiftung Rubel, Zurich).

As used herein, “plant” includes whole plants, plant organs (*e.g.*, fronds (leaves),  
25 stems, roots, *etc.*), seeds, plant cells, and progeny of same. Parts of transgenic plants are to be understood within the scope of the invention to comprise, *e.g.*, plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, tissues, plant calli, embryos as well as flowers, ovules, stems, fruits, leaves, roots, root tips, nodules, and the like originating in transgenic plants or their progeny previously transformed with a polynucleotide of interest  
30 and therefore consisting at least in part of transgenic cells. As used herein, the term “plant cell” includes cells of seeds, embryos, ovules, meristematic regions, callus tissue, leaves, fronds, roots, nodules, shoots, anthers, and pollen.

As used herein, “duckweed nodule” means duckweed tissue comprising duckweed cells where at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of the cells are differentiated cells. As used herein, “differentiated cell,” means a cell with at least one phenotypic characteristic (*e.g.*, a distinctive cell morphology or the expression of a marker nucleic acid or protein) that distinguishes it from undifferentiated cells or from cells found in other tissue types. The differentiated cells of the duckweed nodule culture described herein form a tiled smooth surface of interconnected cells fused at their adjacent cell walls, with nodules that have begun to organize into frond primordium scattered throughout the tissue. The surface of the tissue of the nodule culture has epidermal cells connected to each other via plasmadesmata.

The growth habit of the duckweeds is ideal for culturing methods. The plant rapidly proliferates through vegetative budding of new fronds, in a macroscopic manner analogous to asexual propagation in yeast. This proliferation occurs by vegetative budding from meristematic cells. The meristematic region is small and is found on the ventral surface of the frond. Meristematic cells lie in two pockets, one on each side of the frond midvein. The small midvein region is also the site from which the root originates and the stem arises that connects each frond to its mother frond. The meristematic pocket is protected by a tissue flap. Fronds bud alternately from these pockets. Doubling times vary by species and are as short as 20-24 hours (Landolt (1957) *Ber. Schweiz. Bot. Ges.* 67:271; Chang *et al.* (1977) *Bull. Inst. Chem. Acad. Sin.* 24:19; Datko and Mudd (1970) *Plant Physiol.* 65:16; Venkataraman *et al.* (1970) *Z. Pflanzenphysiol.* 62: 316). Intensive culture of duckweed results in the highest rates of biomass accumulation per unit time (Landolt and Kandeler (1987) *The Family of Lemnaceae—A Monographic Study Vol. 2: Phytochemistry, Physiology, Application, Bibliography* (Veroffentlichungen des Geobotanischen Institutes ETH, Stiftung Rubel, Zurich)), with dry weight accumulation ranging from 6-15% of fresh weight (Tillberg *et al.* (1979) *Physiol. Plant.* 46:5; Landolt (1957) *Ber. Schweiz. Bot. Ges.* 67:271; Stomp, unpublished data). Protein content of a number of duckweed species grown under varying conditions has been reported to range from 15-45% dry weight (Chang *et al.* (1977) *Bull. Inst. Chem. Acad. Sin.* 24:19; Chang and Chui (1978) *Z. Pflanzenphysiol.* 89:91; Porath *et al.* (1979) *Aquatic Botany* 7:272; Appenroth *et al.* (1982) *Biochem. Physiol. Pflanz.* 177:251). Using these values, the level of protein production per liter of medium in duckweed is on the same order of magnitude as yeast gene expression systems.

The present invention also provides transformed microalgae plants expressing an FMDV polypeptide of interest, or fragment or variant thereof. The term “microalgae” or “microalga” refers to members of the family *Thraustochytriaceae*. This family currently is divided into four genera: *Schizochytrium*, *Thraustochytrium*, *Labyrinthuloides*, and  
5 *Japonochytrium*.

The transformed duckweed plants or microalgae of the invention can be obtained by introducing an expression construct comprising a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof, into the duckweed plant or microalga of interest.

10 The term “introducing” in the context of a polynucleotide, for example, an expression construct comprising a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof, is intended to mean presenting to the duckweed plant or microalga the polynucleotide in such a manner that the polynucleotide gains access to the interior of a cell of the duckweed plant or microalga. Where more than one polynucleotide is  
15 to be introduced, these polynucleotides can be assembled as part of a single nucleotide construct, or as separate nucleotide constructs, and can be located on the same or different transformation vectors. Accordingly, these polynucleotides can be introduced into the duckweed or microalga host cell of interest in a single transformation event, in separate transformation events, or, for example, as part of a breeding protocol. The compositions and  
20 methods of the invention do not depend on a particular method for introducing one or more polynucleotides into a duckweed plant or microalga, only that the polynucleotide(s) gains access to the interior of at least one cell of the duckweed plant or microalga. Methods for introducing polynucleotides into plants or algae are known in the art including, but not limited to, transient transformation methods, stable transformation methods, and virus-  
25 mediated methods.

“Transient transformation” in the context of a polynucleotide such as a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof, is intended to mean that a polynucleotide is introduced into the duckweed plant or microalga and does not integrate into the genome of the duckweed plant or microalga.

30 By “stably introducing” or “stably introduced” in the context of a polynucleotide (such as a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof) introduced into a duckweed plant or microalga is intended the introduced

polynucleotide is stably incorporated into the duckweed or microalga genome, and thus the duckweed plant or microalga is stably transformed with the polynucleotide.

“Stable transformation” or “stably transformed” is intended to mean that a polynucleotide, for example, a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof, introduced into a duckweed plant or microalga integrates into the genome of the plant or alga and is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations. In some embodiments, successive generations include progeny produced vegetatively (i.e., asexual reproduction), for example, with clonal propagation. In other embodiments, successive generations include progeny produced via sexual reproduction.

An expression construct comprising a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof, can be introduced into a duckweed plant or microalga of interest using any transformation protocol known to those of skill in art. Suitable methods of introducing nucleotide sequences into duckweed plants or plant cells or nodules or microalgae include microinjection (Crossway *et al.* (1986) *Biotechniques* 4:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606), *Agrobacterium*-mediated transformation (U.S. Patent Nos. 5,563,055 and 5,981,840, both of which are herein incorporated by reference), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722), ballistic particle acceleration (see, *e.g.*, U.S. Patent Nos. 4,945,050; 5,879,918; 5,886,244; and 5,932,782 (each of which is herein incorporated by reference); and Tomes *et al.* (1995) “Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment,” in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe *et al.* (1988) *Biotechnology* 6:923-926). The cells that have been transformed may be grown into plants in accordance with conventional ways.

As noted above, stably transformed duckweed or microalgae can be obtained by any gene transfer method known in the art, such as one of the gene transfer methods disclosed in U.S. Patent No. 6,040,498 or U.S. Patent Application Publication Nos. 2003/0115640, 2003/0033630 or 2002/0088027. Duckweed plant or nodule cultures or microalga can be efficiently transformed with an expression cassette containing a nucleic acid sequence as described herein by any one of a number of methods including *Agrobacterium*-mediated gene transfer, ballistic bombardment or electroporation. The *Agrobacterium* used can be *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. Stable duckweed or microalga

transformants can be isolated by transforming the duckweed or microalga cells with both the nucleic acid sequence of interest and a gene that confers resistance to a selection agent, followed by culturing the transformed cells in a medium containing the selection agent. *See*, for example, U.S. Patent No. 6,040,498, the contents of which are herein incorporated by  
5 reference in their entirety.

The stably transformed duckweed plants or microalgae utilized in these methods should exhibit normal morphology and be fertile by sexual reproduction and/or able to reproduce vegetatively (i.e., asexual reproduction), for example, with clonal propagation. Preferably, transformed duckweed plants or microalgae of the present invention contain a  
10 single copy of the transferred nucleic acid comprising a polynucleotide encoding an antigenic FMDV polypeptide, or fragment or variant thereof, and the transferred nucleic acid has no notable rearrangements therein. It is recognized that the transformed duckweed plants or microalgae of the invention may contain the transferred nucleic acid present in low copy numbers (i.e., no more than twelve copies, no more than eight copies, no more than five  
15 copies, alternatively, no more than three copies, as a further alternative, fewer than three copies of the nucleic acid per transformed cell).

Transformed plants or microalgae expressing an antigenic FMDV polypeptide, or fragment or variant thereof, can be cultured under suitable conditions for expressing the antigenic FMDV polypeptide, or fragment or variant thereof. The antigenic FMDV  
20 polypeptide, or fragment or variant thereof, can then be harvested from the duckweed plant or microalgae, the culture medium, or the duckweed plant or microalgae and the culture medium, and, where desired, purified using any conventional isolation and purification method known in the art, as described elsewhere herein. The antigenic FMDV polypeptide, or fragment or variant thereof, can then be formulated as a vaccine for therapeutic applications,  
25 as described elsewhere herein.

#### Methods of Preparing an FMDV Polypeptide

As described fully herein, in an embodiment, a method of producing an FMDV polypeptide comprises: (a) culturing within a duckweed or microalgae culture medium a duckweed or microalga culture, wherein the duckweed or microalga culture is stably  
30 transformed to express the polypeptide, and wherein the polypeptide is expressed from a nucleotide sequence comprising a coding sequence for said polypeptide; and (b) collecting the antigenic polypeptide from said culture medium. The term collecting includes, but is not limited to, harvesting from the culture medium or purifying.



After production of the recombinant polypeptide in duckweed or microalgae, any method available in the art may be used for protein purification. The various steps include freeing the protein from the nonprotein or plant or microalga material, followed by the purification of the protein of interest from other proteins. Initial steps in the purification process include centrifugation, filtration or a combination thereof. Proteins secreted within the extracellular space of tissues can be obtained using vacuum or centrifugal extraction. Minimal processing could also involve preparation of crude products. Other methods include maceration and extraction in order to permit the direct use of the extract.

Such methods to purify the protein of interest can exploit differences in protein size, physicochemical properties, and binding affinity. Such methods include chromatography, including procainamide affinity, size exclusion, high pressure liquid, reversed-phase, and anion-exchange chromatography, affinity tags, filtration, etc. In particular, immobilized Ni-ion affinity chromatography can be used to purify the expressed protein. See, Favacho *et al.* (2006) *Protein expression and purification* 46:196-203. See also, Zhou *et al.* (2007) *The Protein J* 26:29-37; Wang *et al.* (2006) *Vaccine* 15:2176-2185; and WO/2009/076778; all of which are herein incorporated by reference. Protectants may be used in the purification process such as osmotica, antioxidants, phenolic oxidation inhibitors, protease inhibitors, and the like.

## 20 Methods of Use

In an embodiment, the subject matter disclosed herein is directed to a method of vaccinating an ovine, bovine, caprine, or porcine comprising administering to the ovine, bovine, caprine, or porcine an effective amount of a vaccine which may comprise an effective amount of a recombinant FMDV antigen and a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle.

In one embodiment of the present invention, the method comprises a single administration of a vaccine composition formulated with an emulsion according to the invention. For example, in one embodiment, the immunological or vaccine composition comprises duckweed-expressed FMDV antigens, including polypeptides and VLPs (virus-like particles). Electron microscopy indicates the duckweed transformed with MerE expression vectors likely produce FMDV VLP, and so immunological or vaccine compositions according to the instant invention encompass those comprising FMDV VLP.

In an embodiment, the subject matter disclosed herein is directed to a method of vaccinating an ovine, bovine, caprine, or porcine comprising administering to the ovine, bovine, caprine, or porcine an ovine, bovine, caprine, or porcine FMDV antigen produced in a plant or alga, and plant material from the genus *Lemna* or microalga material from  
5 *schizochytrium*.

In an embodiment, the subject matter disclosed herein is directed to a method of eliciting an immune response comprising administering to the ovine, bovine, caprine, or porcine a vaccine comprising an ovine, bovine, caprine, or porcine FMDV antigen expressed in a plant or alga, wherein an immune response is elicited.

10 In an embodiment, the subject matter disclosed herein is directed to a method of eliciting an immune response comprising administering to the ovine, bovine, caprine, or porcine a vaccine comprising an ovine, bovine, caprine, or porcine FMDV antigen produced in a plant or alga, and plant material from the genus *Lemna* or microalga material from *schizochytrium*., wherein an immune response is elicited.

15 In an embodiment, the subject matter disclosed herein is directed to a method of preparing a stably transformed duckweed plant or microalga culture comprising, (a) introducing into the plant or microalga a genetic construct comprising an FMDV antigen gene; and (b) cultivating the plant or microalga. Methods for transformation of duckweed or microalga are available in the art.

20 In an embodiment, the subject matter disclosed herein is directed to a method of preparing a vaccine or composition comprising isolating an FMDV antigen produced by a duckweed or microalgal expression system and optionally combining with a pharmaceutically or veterinarily acceptable carrier, excipient or vehicle.

In an embodiment, the subject matter disclosed herein is directed to a method of  
25 preparing a vaccine or composition comprising combining an FMDV antigen produced by a *Lemna* expression system and plant material from the genus *Lemna* and optionally a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle.

In another embodiment, the subject matter disclosed herein is directed to a method of preparing a vaccine or composition comprising combining an FMDV antigen produced by a  
30 *Schizochytrium* expression system and *Schizochytrium* material and optionally a pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle.

The administering may be subcutaneously or intramuscularly. The administering may be needle free (for example Pigjet or Bioject).

In one embodiment of the invention, a prime-boost regimen can be employed, which is comprised of at least one primary administration and at least one booster administration using at least one common polypeptide, antigen, epitope or immunogen. Typically the immunological composition or vaccine used in primary administration is different in nature  
5 from those used as a booster. However, it is noted that the same composition can be used as the primary administration and the boost. This administration protocol is called “prime-boost”.

A prime-boost according to the present invention can include a recombinant viral vector is used to express an FMDV coding sequence or fragments thereof encoding an  
10 antigenic polypeptide or fragment or variant thereof. Specifically, the viral vector can express an FMDV gene or fragment thereof that encodes an antigenic polypeptide. Viral vector contemplated herein includes, but not limited to, poxvirus [e.g., vaccinia virus or attenuated vaccinia virus, avipox virus or attenuated avipox virus (e.g., canarypox, fowlpox, dovepox, pigeonpox, quailpox, ALVAC, TROVAC; see e.g., US 5,505,941, US 5,494,8070),  
15 raccoonpox virus, swinepox virus, etc.], adenovirus (e.g., human adenovirus, canine adenovirus), herpesvirus (e.g. canine herpesvirus, herpesvirus of turkey, Marek’s disease virus, infectious laryngotracheitis virus, feline herpesvirus, laryngotracheitis virus (ILTV), bovine herpesvirus, swine herpesvirus), baculovirus, retrovirus, etc. In another embodiment, the avipox expression vector may be a canarypox vector, such as, ALVAC. In yet another  
20 embodiment, the avipox expression vector may be a fowlpox vector, such as, TROVAC. The FMDV antigen of the invention to be expressed is inserted under the control of a specific poxvirus promoter, e.g., the entomopoxvirus *Amsacta moorei* 42K promoter (Barcena, Lorenzo et al. 2000), the vaccinia promoter 7.5 kDa (Cochran et al., 1985), the vaccinia promoter I3L (Riviere et al., 1992), the vaccinia promoter HA (Shida, 1986), the cowpox  
25 promoter ATI (Funahashi et al., 1988), the vaccinia promoter H6 (Taylor et al., 1988b; Guo et al., 1989; Perkus et al., 1989), *inter alia*.

In another embodiment, the avipox expression vector may be a canarypox vector, such as, ALVAC. The FMDV antigen, epitope or immunogen may be FMDV P1-3C. The FMDV viral vector may be a canarypox virus such as vCP2186, vCP2181, or vCP2176, or a  
30 fowlpox virus such as vFP2215 (see US 7,527,960).

In another aspect of the prime-boost protocol of the invention, a composition comprising the FMDV antigen of the invention is administered followed by the administration of vaccine or composition comprising a recombinant viral vector that contains

and expresses the FMDV antigen *in vivo*, or an inactivated viral vaccine or composition comprising the FMDV antigen, or a DNA plasmid vaccine or composition that contains or expresses the FMDV antigen. Likewise, a prime-boost protocol may comprise the administration of vaccine or composition comprising a recombinant viral vector that contains  
5 and expresses an FMDV antigen *in vivo*, or an inactivated viral vaccine or composition comprising an FMDV antigen, or a DNA plasmid vaccine or composition that contains or expresses an FMDV antigen, followed by the administration of a composition comprising the FMDV antigen of the invention. It is further noted that both the primary and the secondary administrations may comprise the composition comprising the FMDV antigen of the  
10 invention.

A prime-boost protocol comprises at least one prime-administration and at least one boost administration using at least one common polypeptide and/or variants or fragments thereof. The vaccine used in prime-administration may be different in nature from those used as a later booster vaccine. The prime-administration may comprise one or more  
15 administrations. Similarly, the boost administration may comprise one or more administrations.

The dose volume of compositions for target species that are mammals, e.g., the dose volume of ovine, bovine, caprine or porcine compositions, based on viral vectors, e.g., non-poxvirus-viral-vector-based compositions, is generally between about 0.1 to about 5.0 ml,  
20 between about 0.1 to about 3.0 ml, and between about 0.5 ml to about 2.5 ml.

The efficacy of the vaccines may be tested about 2 to 4 weeks after the last immunization by challenging animals, such as ovine, bovine, caprine or porcines, with a virulent strain of FMDV, advantageously the FMDV *O1 Manisa*, *O1 BFS or Campos*, *A24 Cruzeiro*, *Asia 1 Shamir*, *A Iran'96*, *A22 Iraq*, *SAT2 Saudi Arabia* strains.

25 Still other strains may include FMDV strains A10-61, A5, A12, A24/Cruzeiro, C3/Indaial, O1, C1-Santa Pau, C1-C5, A22/550/Azerbaijan/65, SAT1-SAT3, A, A/TNC/71/94, A/IND/2/68, A/IND/3/77, A/IND/5/68, A/IND/7/82, A/IND/16/82, A/IND/17/77, A/IND/17/82, A/IND/19/76, A/IND/20/82, A/IND/22/82, A/IND/25/81, A/IND/26/82, A/IND/54/79, A/IND/57/79, A/IND/73/79, A/IND/85/79, A/IND/86/79,  
30 A/APA/25/84, A/APN/41/84, A/APS/44/05, A/APS/50/05, A/APS/55/05, A/APS/66/05, A/APS/68/05, A/BIM/46/95, A/GUM/33/84, A/ORS/66/84, A/ORS/75/88, A/TNAn/60/947/Asia/1, A/IRN/05, Asia/IRN/05, O/HK/2001, O/UKG/3952/2001, O/UKG/4141/2001, Asia 1/HNK/CHA/05 (GenBank accession number EF149010, herein

incorporated by reference), Asia I/XJ (Li, ZhiYong *et al.* Chin Sci Bull, 2007), HK/70 (Chin Sci Bull, 2006, 51(17): 2072—2078), O/UKG/7039/2001, O/UKG/9161/2001, O/UKG/7299/2001, O/UKG/4014/2001, O/UKG/4998/2001, O/UKG/9443/2001, O/UKG/5470/2001, O/UKG/5681/2001, O/ES/2001, HKN/2002, O5India, O/BKF/2/92,  
 5 K/37/84/A, KEN/1/76/A, GAM/51/98/A, A10/Holland, O/KEN/1/91, O/IND49/97, O/IND65/98, O/IND64/98, O/IND48/98, O/IND47/98, O/IND82/97, O/IND81/99, O/IND81/98, O/IND79/97, O/IND78/97, O/IND75/97, O/IND74/97, O/IND70/97, O/IND66/98, O/IND63/97, O/IND61/97, O/IND57/98, O/IND56/98, O/IND55/98, O/IND54/98, O/IND469/98, O/IND465/97, O/IND464/97, O/IND424/97, O/IND423/97,  
 10 O/IND420/97, O/IND414/97, O/IND411/97, O/IND410/97, O/IND409/97, O/IND407/97, O/IND399/97, O/IND39/97, O/IND391/97, O/IND38/97, O/IND384/97, O/IND380/97, O/IND37/97, O/IND352/97, O/IND33/97, O/IND31/97, O/IND296/97, O/IND23/99, O/IND463/97, O/IND461/97, O/IND427/98, O/IND28/97, O/IND287/99, O/IND285/99, O/IND282/99, O/IND281/97, O/IND27/97, O/IND278/97, O/IND256/99, O/IND249/99,  
 15 O/IND210/99, O/IND208/99, O/IND207/99, O/IND205/99, O/IND185/99, O/IND175/99, O/IND170/97, O/IND164/99, O/IND160/99, O/IND153/99, O/IND148/99, O/IND146/99, O/SKR/2000, A22/India/17/77.

Further details of these FMDV strains may be found on the European Bioinformatics Information (EMBL-EBI) web pages, and all of the associated nucleotide sequences are  
 20 herein incorporated by reference. The inventors contemplate that all FMDV strains, both herein listed, and those yet to be identified, could be expressed according to the teachings of the present disclosure to produce, for example, effective vaccine compositions. Both homologous and heterologous strains are used for challenge to test the efficacy of the vaccines. The animal may be challenged intradermally, subcutaneously, spray, intra-nasally,  
 25 intra-ocularly, intra-tracheally, and/or orally.

The prime-boost administrations may be advantageously carried out 2 to 6 weeks apart, for example, about 3 weeks apart. According to one embodiment, a semi-annual booster or an annual booster, advantageously using the viral vector-based vaccine, is also envisaged. The animals are advantageously at least 6 to 8 weeks old at the time of the first  
 30 administration.

The compositions comprising the recombinant antigenic polypeptides of the invention used in the prime-boost protocols are contained in a pharmaceutically or veterinary acceptable vehicle, diluent or excipient. The protocols of the invention protect the animal

from ovine, bovine, caprine or porcine FMDV and/or prevent disease progression in an infected animal.

The various administrations are preferably carried out 1 to 6 weeks apart, and more particularly about 3 weeks apart. According to a preferred mode, an annual booster, preferably using the viral vector-based immunological composition of vaccine, is also  
5 envisaged. The animals are preferably at least one-day-old at the time of the first administration.

It should be understood by one of skill in the art that the disclosure herein is provided by way of example and the present invention is not limited thereto. From the disclosure  
10 herein and the knowledge in the art, the skilled artisan can determine the number of administrations, the administration route, and the doses to be used for each injection protocol, without any undue experimentation.

The present invention contemplates at least one administration to an animal of an efficient amount of the therapeutic composition made according to the invention. The animal  
15 may be male, female, pregnant female and newborn. This administration may be via various routes including, but not limited to, intramuscular (IM), intradermal (ID) or subcutaneous (SC) injection or via intranasal or oral administration. The therapeutic composition according to the invention can also be administered by a needleless apparatus (as, for example with a Pigjet, Dermojet, Biojector, Avijet (Merial, GA, USA), Vetjet or Vitajet apparatus (Bioject,  
20 Oregon, USA)). Another approach to administering plasmid compositions is to use electroporation (see, e.g. Tollefsen et al., 2002; Tollefsen et al., 2003; Babiuk et al., 2002; PCT Application No. WO99/01158). In another embodiment, the therapeutic composition is delivered to the animal by gene gun or gold particle bombardment.

In one embodiment, the invention provides for the administration of a therapeutically  
25 effective amount of a formulation for the delivery and expression of an FMDV antigen or epitope in a target cell. Determination of the therapeutically effective amount is routine experimentation for one of ordinary skill in the art. In one embodiment, the formulation comprises an expression vector comprising a polynucleotide that expresses an FMDV antigen or epitope and a pharmaceutically or veterinarily acceptable carrier, vehicle or excipient. In  
30 another embodiment, the pharmaceutically or veterinarily acceptable carrier, vehicle or excipient facilitates transfection or other means of transfer of polynucleotides to a host animal and/or improves preservation of the vector or protein in a host.

In one embodiment, the subject matter disclosed herein provides a detection method for differentiation between infected and vaccinated animals (DIVA).

It is disclosed herein that the use of the vaccine or composition of the present invention allows the detection of FMDV infection in an animal. It is disclosed herein that the use of the vaccine or composition of the present invention allows the detection of the infection in animals by differentiating between infected and vaccinated animals (DIVA). A method is disclosed herein for diagnosing the infection of FMDV in an animal using an FMDV non-structural protein (e.g. a FMDV 3ABC or 3D-specific ELISA).

#### 10 Article of Manufacture

In an embodiment, the subject matter disclosed herein is directed to a kit for performing a method of eliciting or inducing an immune response which may comprise any one of the recombinant FMDV immunological compositions or vaccines, or inactivated FMDV immunological compositions or vaccines, recombinant FMDV viral compositions or vaccines, and instructions for performing the method.

Another embodiment of the invention is a kit for performing a method of inducing an immunological or protective response against FMDV in an animal comprising a composition or vaccine comprising an FMDV antigen of the invention and a recombinant FMDV viral immunological composition or vaccine, and instructions for performing the method of delivery in an effective amount for eliciting an immune response in the animal.

Another embodiment of the invention is a kit for performing a method of inducing an immunological or protective response against FMDV in an animal comprising a composition or vaccine comprising an FMDV antigen of the invention and an inactivated FMDV immunological composition or vaccine, and instructions for performing the method of delivery in an effective amount for eliciting an immune response in the animal.

Yet another aspect of the present invention relates to a kit for prime-boost vaccination according to the present invention as described above. The kit may comprise at least two vials: a first vial containing a vaccine or composition for the prime-vaccination according to the present invention, and a second vial containing a vaccine or composition for the boost-vaccination according to the present invention. The kit may advantageously contain additional first or second vials for additional primo-vaccinations or additional boost-vaccinations.

The following embodiments are encompassed by the invention. In an embodiment, a composition comprising an FMDV antigen or fragment or variant thereof and a pharmaceutical or veterinarily acceptable carrier, excipient, or vehicle is disclosed. In another embodiment, the composition described above wherein the FMDV antigen or fragment or variant thereof comprises an immunogenic fragment comprising at least 15 amino acids of an ovine, bovine, caprine, or porcine FMDV antigen is disclosed. In yet another embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is produced in duckweed or microalgae are disclosed. In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is partially purified are disclosed. In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is substantially purified are disclosed.

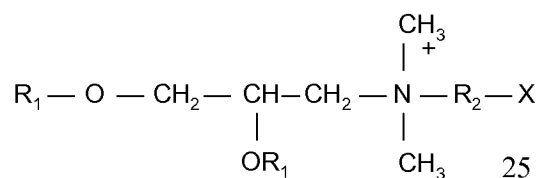
In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof is an ovine, bovine, caprine, or porcine FMDV polypeptide are disclosed. In an embodiment, the above compositions wherein the FMDV polypeptide is a P1-3C polypeptide, P1 polypeptide, VP0 polypeptide, VP1 polypeptide, VP3 polypeptide, VP2 polypeptide, VP4 polypeptide, 2A polypeptide, 2B1 polypeptide, or 3C polypeptide are disclosed. In an embodiment, the above compositions wherein the FMDV antigen or fragment or variant thereof has at least 80% sequence identity to the sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, 29 are disclosed. In one embodiment, the above compositions wherein the FMDV antigen is encoded by a polynucleotide having at least 70% sequence identity to the sequence as set forth in SEQ ID NOs:1, 2, 9, 11, 13, 14, 15, 16, 18, 19, 21, 22, or 24, 25, 27, 28, 30-35 are disclosed. In an embodiment, the above compositions wherein the pharmaceutical or veterinarily acceptable carrier, excipient, or vehicle is a water-in-oil emulsion or an oil-in-water emulsion are disclosed. In another embodiment, a method of vaccinating an animal susceptible to ovine, bovine, caprine, or porcine FMDV comprising administering the compositions above to the animal is disclosed. In an embodiment, a method of vaccinating an animal susceptible to ovine, bovine, caprine, or porcine FMDV comprising a prime-boost regime is disclosed. In an embodiment, a substantially purified antigenic polypeptide expressed in duckweed or microalga, wherein the polypeptide comprises: an amino acid sequence having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, 29 is disclosed. In any embodiment the animal is preferably an ovine, a bovine, a porcine, or a caprine. In one embodiment, a method of diagnosing FMDV infection in an animal is disclosed. In yet



another embodiment, a kit for prime-boost vaccination comprising at least two vials, wherein a first vial containing the composition of the present invention, and a second vial containing a composition for the boost-vaccination comprising a composition comprising a recombinant viral vector, or a composition comprising an inactivated viral composition, or a DNA plasmid composition that contains or expresses the FMDV antigen is disclosed.

The pharmaceutically or veterinarily acceptable carriers or vehicles or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or vehicle or excipient can be a 0.9% NaCl (e.g., saline) solution or a phosphate buffer. Other pharmaceutically or veterinarily acceptable carrier or vehicle or excipients that can be used for methods of this invention include, but are not limited to, poly-(L-glutamate) or polyvinylpyrrolidone. The pharmaceutically or veterinarily acceptable carrier or vehicle or excipients may be any compound or combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector *in vitro*); advantageously, the carrier, vehicle or excipient may facilitate transfection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

The cationic lipids containing a quaternary ammonium salt which are advantageously but not exclusively suitable for plasmids, are advantageously those having the following formula:



in which R1 is a saturated or unsaturated straight-chain aliphatic radical having 12 to 18 carbon atoms, R2 is another aliphatic radical containing 2 or 3 carbon atoms and X is an amine or hydroxyl group, e.g. the DMRIE. In another embodiment the cationic lipid can be associated with a neutral lipid, e.g. the DOPE.

Among these cationic lipids, preference is given to DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propane ammonium; WO96/34109), advantageously associated with a neutral lipid, advantageously DOPE (dioleoyl-phosphatidyl-ethanol amine; Behr, 1994), to form DMRIE-DOPE.

Advantageously, the plasmid mixture with the adjuvant is formed extemporaneously and advantageously contemporaneously with administration of the preparation or shortly before administration of the preparation; for instance, shortly before or prior to administration, the plasmid-adjuvant mixture is formed, advantageously so as to give enough  
5 time prior to administration for the mixture to form a complex, e.g. between about 10 and about 60 minutes prior to administration, such as approximately 30 minutes prior to administration.

When DOPE is present, the DMRIE:DOPE molar ratio is advantageously about 95: about 5 to about 5: about 95, more advantageously about 1: about 1, e.g., 1:1.

10 The DMRIE or DMRIE-DOPE adjuvant:plasmid weight ratio can be between about 50: about 1 and about 1: about 10, such as about 10: about 1 and about 1: about 5, and about 1: about 1 and about 1: about 2, e.g., 1:1 and 1:2.

In another embodiment, pharmaceutically or veterinarily acceptable carrier, excipient, or vehicle may be a water-in-oil emulsion. Examples of suitable water-in-oil emulsions  
15 include oil-based water-in-oil vaccinal emulsions which are stable and fluid at 4°C containing: from 6 to 50 v/v% of an antigen-containing aqueous phase, preferably from 12 to 25 v/v%, from 50 to 94 v/v% of an oil phase containing in total or in part a non-metabolizable oil (e.g., mineral oil such as paraffin oil) and/or metabolizable oil (e.g., vegetable oil, or fatty acid, polyol or alcohol esters), from 0.2 to 20 p/v% of surfactants,  
20 preferably from 3 to 8 p/v%, the latter being in total or in part, or in a mixture either polyglycerol esters, said polyglycerol esters being preferably polyglycerol (poly)ricinoleates, or polyoxyethylene ricin oils or else hydrogenated polyoxyethylene ricin oils. Examples of surfactants that may be used in a water-in-oil emulsion include ethoxylated sorbitan esters (e.g., polyoxyethylene (20) sorbitan monooleate (TWEEN 80®), available from AppliChem,  
25 Inc., Cheshire, CT) and sorbitan esters (e.g., sorbitan monooleate (SPAN 80®), available from Sigma Aldrich, St. Louis, MO). In addition, with respect to a water-in-oil emulsion, see also US Patent No. 6,919,084, e.g., Example 8 thereof, incorporated herein by reference. In some embodiments, the antigen-containing aqueous phase comprises a saline solution comprising one or more buffering agents. An example of a suitable buffering solution is  
30 phosphate buffered saline. In an advantageous embodiment, the water-in-oil emulsion may be a water/oil/water (W/O/W) triple emulsion (U.S. Patent No. 6,358,500). Examples of other suitable emulsions are described in U.S. Patent No. 7,371,395.

The immunological compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as  
5 oligodeoxyribonucleotide sequences having one or more non-methylated CpG units (Klinman et al., 1996; WO98/16247), (3) an oil in water emulsion, such as the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on page 183 of the same work, (4) cation lipids containing a quaternary ammonium salt, e.g., DDA (5)  
10 cytokines, (6) aluminum hydroxide or aluminum phosphate, (7) saponin or (8) other adjuvants discussed in any document cited and incorporated by reference into the instant application, or (9) any combinations or mixtures thereof.

The oil in water emulsion (3), which is especially appropriate for viral vectors, can be based on: light liquid paraffin oil (European pharmacopoeia type), isoprenoid oil such as  
15 squalane, squalene, oil resulting from the oligomerization of alkenes, e.g. isobutene or decene, esters of acids or alcohols having a straight-chain alkyl group, such as vegetable oils, ethyl oleate, propylene glycol, di(caprylate/caprate), glycerol tri(caprylate/caprate) and propylene glycol dioleate, or esters of branched, fatty alcohols or acids, especially isostearic acid esters.

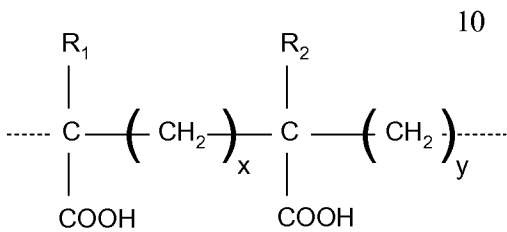
20 The oil is used in combination with emulsifiers to form an emulsion. The emulsifiers may be nonionic surfactants, such as: esters of on the one hand sorbitan, mannide (e.g. anhydromannitol oleate), glycerol, polyglycerol or propylene glycol and on the other hand oleic, isostearic, ricinoleic or hydroxystearic acids, said esters being optionally ethoxylated, or polyoxypropylene-polyoxyethylene copolymer blocks, such as Pluronic, e.g., L121.

25 Among the type (1) adjuvant polymers, preference is given to polymers of crosslinked acrylic or methacrylic acid, especially crosslinked by polyalkenyl ethers of sugars or polyalcohols. These compounds are known under the name carbomer (Pharmeuropa, vol. 8, no. 2, June 1996). One skilled in the art can also refer to U.S. Patent No. 2,909,462, which provides such acrylic polymers crosslinked by a polyhydroxyl compound having at least three  
30 hydroxyl groups, preferably no more than eight such groups, the hydrogen atoms of at least three hydroxyl groups being replaced by unsaturated, aliphatic radicals having at least two carbon atoms. The preferred radicals are those containing 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals can also contain

other substituents, such as methyl. Products sold under the name Carbopol (BF Goodrich, Ohio, USA) are especially suitable. They are crosslinked by allyl saccharose or by allyl pentaerythritol. Among them, reference is made to Carbopol 974P, 934P and 971P.

As to the maleic anhydride-alkenyl derivative copolymers, preference is given to EMA (Monsanto), which are straight-chain or crosslinked ethylene-maleic anhydride copolymers and they are, for example, crosslinked by divinyl ether. Reference is also made to J. Fields et al., 1960.

With regard to structure, the acrylic or methacrylic acid polymers and EMA are preferably formed by basic units having the following formula:



15 in which:

- R1 and R2, which can be the same or different, represent H or CH3
- x = 0 or 1, preferably x = 1
- y = 1 or 2, with x + y = 2.

For EMA, x = 0 and y = 2 and for carbomers x = y = 1.

20 These polymers are soluble in water or physiological salt solution (20 g/l NaCl) and the pH can be adjusted to 7.3 to 7.4, e.g., by soda (NaOH), to provide the adjuvant solution in which the expression vector(s) can be incorporated. The polymer concentration in the final immunological or vaccine composition can range between about 0.01 to about 1.5% w/v, about 0.05 to about 1% w/v, and about 0.1 to about 0.4% w/v.

25 The cytokine or cytokines (5) can be in protein form in the immunological or vaccine composition, or can be co-expressed in the host with the immunogen or immunogens or epitope(s) thereof. Preference is given to the co-expression of the cytokine or cytokines, either by the same vector as that expressing the immunogen or immunogens or epitope(s) thereof, or by a separate vector thereof.

30 The invention comprehends preparing such combination compositions; for instance by admixing the active components, advantageously together and with an adjuvant, carrier, cytokine, and/or diluent.

Cytokines that may be used in the present invention include, but are not limited to, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), interferon  $\alpha$  (IFN $\alpha$ ), interferon  $\beta$  (IFN $\beta$ ), interferon  $\gamma$ , (IFN $\gamma$ ), interleukin-1 $\alpha$ (IL-1 $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4  
5 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), tumor necrosis factor  $\beta$  (TNF $\beta$ ), and transforming growth factor  $\beta$  (TGF $\beta$ ). It is understood that cytokines can be co-administered and/or sequentially administered with the immunological or vaccine composition of the present invention. Thus,  
10 for instance, the vaccine of the instant invention can also contain an exogenous nucleic acid molecule that expresses *in vivo* a suitable cytokine, e.g., a cytokine matched to this host to be vaccinated or in which an immunological response is to be elicited (for instance, a bovine cytokine for preparations to be administered to bovines).

Advantageously, the immunological composition and/or vaccine according to the  
15 invention comprise or consist essentially of or consist of an effective quantity to elicit a therapeutic response of one or more Duckweed-expressed polypeptides as discussed herein; and, an effective quantity can be determined from this disclosure, including the documents incorporated herein, and the knowledge in the art, without undue experimentation.

In the case of immunological composition and/or vaccine based on a Duckweed-  
20 expressed polypeptides, a dose may include, about in 1  $\mu\text{g}$  to about 2000  $\mu\text{g}$ , advantageously about 50  $\mu\text{g}$  to about 1000  $\mu\text{g}$  and more advantageously from about 100  $\mu\text{g}$  to about 500  $\mu\text{g}$  of FMDV antigen, epitope or immunogen. The dose volumes can be between about 0.1 and about 10 ml, advantageously between about 0.2 and about 5 ml.

The invention will now be further described by way of the following non-limiting  
25 examples.

**EXAMPLES**

Construction of DNA inserts, plasmids and recombinant viral or plant vectors was carried out using the standard molecular biology techniques described by J. Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989).

**Example 1 Generation and screening of FMDV-expressing duckweed lines**

Duckweed optimized FMDV P1 and 3C sequences were produced and cloned into the parental plasmid to generate the MerE vectors depicted in FIGs. 6-9. Four independent constructs were designed for the FMDV project. Table 3 summarizes the number of transgenic lines that were generated and screened and FIG. 3 provides a schematic representation of gene structure for the FMDV inserts. Three lines express the P1 capsid + 3C protease (MerE01, 3, 4), whereas the other express the P1 capsid antigen alone (MerE02). ELISA and Agilent analyses were used to quantify the expression of the FMDV antigens. Western blots were performed to verify the correct size of expressed proteins (FIG. 12). The highest FMDV serotype A24-expressing duckweed lines, as determined by mRNA analysis and by western blot, were grown in scale vessels to provide biomass for use in characterization and animal studies.

Table 3: Line generation and further screening of FMDV “MerE” lines

<b>Construct</b>	<b>Description</b>	<b># of lines generated</b>	<b># of lines screened</b>
MerE01	P1-3C	16	16
MerE02	P1 alone (Optimized 5' UTR)	100	100
MerE03	P1-2A + 3C	20	20
MerE04	P1-2A + 3C (Optimized 5' UTR)	8	8

*Screening.* 144 transgenic FMDV lines were developed. After 144 FMDV expressing lines were developed, they were screened to determine the relative levels of FMDV

expression in the tissue. FMDV mRNA levels were measured via RNA dot blot (FIG. 10) and real time rtPCR (FIG. 11). FMDV proteins levels were measured using western blot and ELISA. FMDV-expressing duckweed lines were screened via RNA dot blot using a labeled P1 region to probe the blot. High expressing lines were confirmed by real time rtPCR, and there was reasonable agreement between the mRNA quantification methods. The results indicate that the FMDV P1 is highly expressed in duckweed.

*Methods.* The duckweed plants were grown for two weeks in small research vessels and the resulting tissue was collected and snap frozen in liquid N<sub>2</sub>. Total RNA was extracted from 100 mg of frozen tissue samples using the Qiagen RNeasy 96-well RNA extraction kit (Qiagen, Valencia, CA, #74181). RNA was quantified, vacuum transferred to nylon membranes and probed with a gene fragment from the P1 region of the FMDV gene sequence. Crude tissue extraction from lines containing FMDV antigens were prepared. All steps were taken place at 4°C. One hundred grams of frozen biomass was mixed with 200ml extraction buffer (50mM NaPO<sub>4</sub>, 0.3M NaCl, 10mm EDTA, pH 7.4) and then homogenized in a Waring Blender with a 20 second burst for 4 times and 10-20 seconds cooling in between. The homogenate was centrifuged at 10,000 x g for 30 min at 4°C, clarified by passing through a cheese cloth to remove any large debris and finally cellulose acetate filter (0.22µm). The resulting homogenate was stored at 4°C or on ice for immediate testing. The remaining homogenate was frozen in aliquots at -80°C for further analysis. Total soluble protein (TSP) was determined using the Bradford assay with bovine serum albumin as a standard. Protein level analysis was performed for P1-expressing duckweed lines. Duplicate samples were read from two separate extractions (total of 4 measurements).

*RNA Results.* A broad range of RNA expression levels was observed between the various FMDV transgenic lines (FIG. 11). RNA expression from transgenic lines showing the strongest hybridization signals were then confirmed by real time rtPCR. Relative expression of these lines was shown to be comparable between the dot-blot and PCR methods. The top lines were then selected for further western blot characterization (FIG. 12).

*Protein Results.* The ELISA results are summarized in Table 4 and the densitometry and Agilent results are summarized in Table 5. FIG. 12 depicts the WB results, with arrows indicating the FMDV bands. Lane 1 (both gels) - marker, 2 (both gels) – sucrose-purified FMDV A24 inactivated virus, 3 (both gels) - duckweed wild type lysate. Lanes 4-6 of MerE01-expressing duckweed lysates (three lines - 6, 8 and 10). Lanes 4-12 MerE02-expressing duckweed lysates (nine lines - 2, 3, 16, 20, 23, 24, 25, 26 and 31). MerE01-

expressing duckweed lines 6 and 10 appear to produce a properly cleaved protein (this line contains the 3C protease). MerE02-expressing duckweed lines (lacking the protease) appear to produce significant amounts of the uncleaved P1 protein, as well as higher MW aggregate species.

5

Table 4: ELISA Quantification Results for the MerE01 Lines.

Duckweed line	Average Antigen Conc. ( $\mu\text{g/ml}$ )	Average TSP (mg/ml)	% TSP
MerE01-6	11.35-16.84	5.0	0.23-0.34
MerE01-10	0.79-3.4	5.0	0.02-0.07

Table 5: Expression Level of Duckweed-FMDV MerE01 Lines.

Duckweed line	Average Antigen Conc. ( $\mu\text{g/ml}$ ) <sup>1</sup>	Average%TSP <sup>2</sup>
MerE02-2	54.6 $\pm$ 4.3	2.35
MerE02-3	36.8 $\pm$ 6.6	1.70
MerE02-26	91.6 $\pm$ 9.0	3.95

<sup>1</sup>WT background band at the same MW (around 99-100 kDa) less than 5  $\mu\text{g/ml}$ .

10 <sup>2</sup>Average total soluble protein between 2.1 and 2.3 mg/ml by Agilent analysis.

Inventors observed expression of the 3C protease was exerting toxic effects on the recombinant duckweed, causing reduced growth and antigen production, as compared to, for example, recombinant duckweed expressing only the P1 polypeptide. Therefore, new constructs were produced, some of which comprised a 3C expression cassette driven by an inducible promoter. Unhindered by the toxic effects of 3C expression, the duckweed grew and expressed robust levels of P1. Then, after the culture reached an optimum density, 3C expression was induced so it could cleave the established pools of P1 into the various subunits. The subunits were then able to assemble into protomers, pentamers, and finally, the FMD viral particles (schematized in FIG. 16).

20 These new FMDV duckweed expression constructs included MerF01 (SEQ ID NO:30), which incorporated P1 – 3C expressed driven by the Super Promoter in standard single gene expression vector (EC1.0, analogous to approach taken to produce MerE01).



MerF02 (SEQ ID NO:31) used the *Lemna gibba* NPR promoter (ABA inducible promoter) to express P1 – 3C and MerF03 (SEQ ID NO:32) expressed P1-2A and 3C with separate promoters in a single vector: P1 – 2A expression driven by Super Promoter and 3C expression driven by *Lemna minor* R-histone promoter. MerF04 (SEQ ID NO:33) expressed  
5 P1-2A driven by Super promoter and expressed 3C driven by *Lemna gibba* NPR promoter. MerF05 (SEQ ID NO:34) expressed P1-2A (SpUbq promoter) and 3C (*Lemna minor* R-histone promoter), and MerF06 (SEQ ID NO:35) expressed P1-2A (SpUbq promoter) and 3C (*Lemna gibba* NPR promoter).

*Antigen preparation.* Crude extract was produced using a Waring Blender or bead  
10 beater (1:4 biomass to buffer ratio, PBS pH7.2), and the lysates were clarified by centrifugation (~10K x g). To produce the concentrate, the clarified lysate was filtered through a sterile 0.22 µm filter. This material was then concentrated using 30KDa centricon filters (5-10x), and subjected to *in vitro* characterization or animal study.

#### **Example 2** - Expression of FMDV antigens in *Schizochytrium*

15 Codon-optimized FMDV P1 and 3C genes are cloned into the expression vector pAB0018 (ATCC deposit no. PTA9616). The specific nucleic acid sequence of FMDV gene is optimized for expression in *Schizochytrium* sp. Additionally, the expression vector contains a selection marker cassette conferring resistance to *Schizochytrium* transformants, a promoter from the *Schizochytrium* native gene to drive expression of the transgene, and a terminator.

20 *Schizochytrium* sp. (ATCC 20888) is used as a host for transformation with the expression vector containing the FDMV gene using electroporation method. Cryostocks of transgenic strains of *Schizochytrium* are grown in M50-20 (described in US 2008/0022422) to confluency. The propagated *Schizochytrium* cultures are transferred to 50mL conical tubes and centrifuged at 3000g for 15min or 100,000g for 1hour. The resulting pellet and the  
25 soluble fraction are used for expression analysis and in animal challenge study.

#### **Example 3** - Vaccination of pigs – safety assessment

Three (3) groups of five (5) pigs were vaccinated on days 0 and 21 (D0 and D21) according to the study design (Table 6). Details of the TS6 adjuvant (emulsions) may be found in US patent numbers US 7,608,279 B2 and US 7,371,395 B2 (both to Merial  
30 Limited).

*Assessment of Safety.* No adverse general/systemic reactions were observed after vaccination, though transient, slight to moderate increases of rectal temperature were observed in all groups. Locally, slight to moderate reactions were observed for the duckweed groups. The vaccines were globally acceptable for all groups.

5 Table 6 – Vaccination of pigs – study design

Group	Antigen	Dilution	Adjuvant
G1	MerE01	Not diluted	TS6
G2	MerE01	1/10 diluted	TS6
G3	-	Control	-

**Example 4 – Vaccination of Cattle**

Seventeen (17) conventional cattle, free from FMDV, but not previously immunized against FMDV, were used for this study (study design summarized in Table 7). At D-1, the cattle were allocated into to three groups of 5 animals, and 1 group of 2 animals. Vaccination was performed on D0, via sub-cutaneous route on the left side of the neck. Challenge was administered on D21, and the final observations were made on D29. Tested vaccines expressing FMDV A24 P1-3C were formulated in TS6 adjuvant as described above. Separate vials of antigens and adjuvant were stored at 5 °C prior to administration. The contents of the vials were reconstituted extemporaneously by mixing antigen with adjuvant accordingly. The volume of a dose of the reconstituted vaccines was 2 mL. The challenge strain was FMD type A24 virus prepared to obtain 10 000 ID50 per 0.2 mL. The challenge strain was diluted in Hanks MEM 2% fetal bovine serum with antibiotics.

20 Table 7 – cattle vaccine summary

Group	Antigen	Dose	# Cattle
A	Duckweed expressed FMDV	2 ml	5
B	Experimental recombinant vaccine 1 expressed FMDV	2 ml	5
C	Experimental recombinant vaccine 2 expressed FMDV	2 ml	5
Control	NA	NA	2

On D21, all animals were tranquilized by administration of Xylazine (0.03 – 0.10 mg per kg BW I.V. (0,15 - 0,5 ml per 100 kg BW I.V.) and challenged with 10 000 ID<sub>50</sub> of virus by intra-dermal route, into two locations of the tongue, 0.1 ml per location. The general well  
5 being of the animals was checked daily from D1 to D21. Any clinical observation and treatments administered (commercial name, active ingredient, preemption date, volume, route) were noted.

Necropsy results (Number of feet with at least 1 vesicle, min=0, max=4) for the animals were as follows: duckweed (4, 3, 2, 1, 1), experimental recombinant vaccine 1 (0, 0,  
10 1, 1, 1), experimental recombinant vaccine 2 (3, 4, 4, 4, 4) and the Controls (4, 4). The challenge study was validated since all 2 control cattle showed FMD clinical signs, in addition, experimental vaccine 2 also exhibited clear FMD signs, and can be served as negative control. Even though the cattle in duckweed group were not well protected, clinical symptom was eased in two cattle. There were two cattle in experimental vaccine 1 group  
15 were considered to be protected, the result was consistent with other reports using the similar expression system and suggested that intact VLP was immunogenic and was able to provide protection against virulent FMDV.

It is likely the duckweed vaccine will be improved by 1) increasing percentage of intact VLP, and 2) improving the concentration/purification strategies, so that the antigen will  
20 be more accessible to the immune system in order to achieve greater protection.

#### **Example 5** – characterization of FMDV antigens by sandwich ELISA

*In vitro* characterization of duckweed expressed FMDV was conducted with a sandwich ELISA using FA24 005E9G (monoclonal antibody against FMDV A24) and  
25 biotinylated M3, a 12S-specific llama single chain domain antibody.

Results (FIG. 17) indicated that positive optical density at 450nm/630nm for 1x crude extract, 5x, and 10x concentrated crude extract, which was in good agreement with inactivated FMDV A24 as positive control, whereas crude extract prepared from duckweed wild type remained undetectable. ELISA titer was determined as the decimal logarithm per  
30 milliliter of the dilution for which 50% of the maximum OD was obtained. Table 8 demonstrated that ELISA titer for 5x was higher than 1x extract, however, equivalent to 10x extract, 5x concentrated duckweed crude extract was used in the cattle challenge study.

Table 8. Summary of ELISA titer

<b>Sample</b>	<b>Titre log10OD50/ml</b>
<b>Inactivated FMDV A24</b>	<b>5.46</b>
<b>MerE01 1x crude extract</b>	<b>2.79</b>
<b>MerE01 5x concentrated crude extract</b>	<b>3.34</b>
<b>MerE01 10x concentrated crude extract</b>	<b>3.21</b>
<b>Duckweed wild type 1x crude extract</b>	<b>Negative</b>

\* \* \* \* \*

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

All documents cited or referenced in the application cited documents, and all documents cited or referenced herein (“herein cited documents”), and all documents cited or referenced in herein cited documents, together with any manufacturer’s instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

**REFERENCES**

- Andreansky, S. S., B. He, et al. (1996). "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors." Proc Natl Acad Sci U S A **93**(21): 11313-8.
- 5
- Antoine, G., F. Scheiflinger, et al. (1998). "The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses." Virology **244**(2): 365-96.
- Ballay, A., M. Levrero, et al. (1985). "In vitro and in vivo synthesis of the hepatitis B virus surface antigen and of the receptor for polymerized human serum albumin from recombinant human adenoviruses." Embo J **4**(13B): 3861-5.
- 10
- Barcena, J., M. M. Lorenzo, et al. (2000). "Sequence and analysis of a swinepox virus homologue of the vaccinia virus major envelope protein P37 (F13L)." J Gen Virol **81**(Pt 4): 1073-85.
- 15
- Boshart, M., F. Weber, et al. (1985). "A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus." Cell **41**(2): 521-30.
- Bradel-Tretheway, B. G., Z. Zhen, et al. (2003). "Effects of codon-optimization on protein expression by the human herpesvirus 6 and 7 U51 open reading frame." J Virol Methods **111**(2): 145-56.
- 20
- Carroll, M. W., W. W. Overwijk, et al. (1997). "Highly attenuated modified vaccinia virus Ankara (MVA) as an effective recombinant vector: a murine tumor model." Vaccine **15**(4): 387-94.
- Cochran, M. A., C. Puckett, et al. (1985). "In vitro mutagenesis of the promoter region for a vaccinia virus gene: evidence for tandem early and late regulatory signals." J Virol
- 25
- 54**(1): 30-7.
- De Groot, A. S. and F. G. Rothman (1999). "In silico predictions; in vivo veritas." Nat Biotechnol **17**(6): 533-4.
- Disbrow, G. L., I. Sunitha, et al. (2003). "Codon optimization of the HPV-16 E5 gene enhances protein expression." Virology **311**(1): 105-14.
- 30
- Felgner, J. H., R. Kumar, et al. (1994). "Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations." J Biol Chem **269**(4): 2550-61.
- Frolov, I., T. A. Hoffman, et al. (1996). "Alphavirus-based expression vectors: strategies and applications." Proc Natl Acad Sci U S A **93**(21): 11371-7.

- Funahashi, S., T. Sato, et al. (1988). "Cloning and characterization of the gene encoding the major protein of the A-type inclusion body of cowpox virus." J Gen Virol **69** ( Pt 1): 35-47.
- Geysen, H. M. (1990). "Molecular technology: peptide epitope mapping and the pin  
5 technology." Southeast Asian J Trop Med Public Health **21**(4): 523-33.
- Geysen, H. M., S. J. Barteling, et al. (1985). "Small peptides induce antibodies with a sequence and structural requirement for binding antigen comparable to antibodies raised against the native protein." Proc Natl Acad Sci U S A **82**(1): 178-82.
- Geysen, H. M., R. H. Meloen, et al. (1984). "Use of peptide synthesis to probe viral antigens  
10 for epitopes to a resolution of a single amino acid." Proc Natl Acad Sci U S A **81**(13): 3998-4002.
- Graham, F. L. (1990). "Adenoviruses as expression vectors and recombinant vaccines." Trends Biotechnol **8**(4): 85-7.
- Guo, P. X., S. Goebel, et al. (1989). "Expression in recombinant vaccinia virus of the equine  
15 herpesvirus 1 gene encoding glycoprotein gp13 and protection of immunized animals." J Virol **63**(10): 4189-98.
- Hartikka, J., M. Sawdey, et al. (1996). "An improved plasmid DNA expression vector for direct injection into skeletal muscle." Hum Gene Ther **7**(10): 1205-17.
- Hemmer, B., C. Pinilla, et al. (1998). "The use of soluble synthetic peptide combinatorial  
20 libraries to determine antigen recognition of T cells." J Pept Res **52**(5): 338-45.
- Ju, Q., D. Edelstein, et al. (1998). "Transduction of non-dividing adult human pancreatic beta cells by an integrating lentiviral vector." Diabetologia **41**(6): 736-9.
- Kim, C. H., Y. Oh, et al. (1997). "Codon optimization for high-level expression of human erythropoietin (EPO) in mammalian cells." Gene **199**(1-2): 293-301.
- 25 Kitson, J. D., K. L. Burke, et al. (1991). "Chimeric polioviruses that include sequences derived from two independent antigenic sites of foot-and-mouth disease virus (FMDV) induce neutralizing antibodies against FMDV in guinea pigs." J Virol **65**(6): 3068-75.
- Klinman, D. M., A. K. Yi, et al. (1996). "CpG motifs present in bacteria DNA rapidly induce  
30 lymphocytes to secrete interleukin 6, interleukin 12, and interferon gamma." Proc Natl Acad Sci U S A **93**(7): 2879-83.

- Kwissa, M., K. van Kampen, et al. (2000). "Efficient vaccination by intradermal or intramuscular inoculation of plasmid DNA expressing hepatitis B surface antigen under desmin promoter/enhancer control." Vaccine **18**(22): 2337-44.
- 5 Laval, F., R. Paillot, et al. (2002). "Quantitative analysis of the antigen-specific IFN $\gamma$ + T cell-mediated immune response in conventional outbred pigs: kinetics and duration of the DNA-induced IFN $\gamma$ + CD8+ T cell response." Vet Immunol Immunopathol **90**(3-4): 191-201.
- Luckow, V. A. and M. D. Summers (1988). "Signals important for high-level expression of foreign genes in *Autographa californica* nuclear polyhedrosis virus expression vectors." Virology **167**(1): 56-71.
- 10 Marshall, E., L. B. Woolford, et al. (1997). "Continuous infusion of macrophage inflammatory protein MIP-1 $\alpha$  enhances leucocyte recovery and haemopoietic progenitor cell mobilization after cyclophosphamide." Br J Cancer **75**(12): 1715-20.
- McClements, W. L., M. E. Armstrong, et al. (1996). "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease." Proc Natl Acad Sci U S A **93**(21): 11414-20.
- 15 Miyazaki, J., S. Takaki, et al. (1989). "Expression vector system based on the chicken beta-actin promoter directs efficient production of interleukin-5." Gene **79**(2): 269-77.
- 20 Moss, B. (1996). "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety." Proc Natl Acad Sci U S A **93**(21): 11341-8.
- Paoletti, E. (1996). "Applications of pox virus vectors to vaccination: an update." Proc Natl Acad Sci U S A **93**(21): 11349-53.
- Pearson, W. R. and D. J. Lipman (1988). "Improved tools for biological sequence comparison." Proc Natl Acad Sci U S A **85**(8): 2444-8.
- 25 Pennock, G. D., C. Shoemaker, et al. (1984). "Strong and regulated expression of *Escherichia coli* beta-galactosidase in insect cells with a baculovirus vector." Mol Cell Biol **4**(3): 399-406.
- Perkus, M. E., K. Limbach, et al. (1989). "Cloning and expression of foreign genes in vaccinia virus, using a host range selection system." J Virol **63**(9): 3829-36.
- 30 Powell, M. F. and M. J. Newman (1995). Vaccine Design, The Subunit and Adjuvant Approach. A Compendium of Vaccine Adjuvants and Excipients. F. Vogel and M. Powell. New York, Plenum Press. **6**: 147, 183.

- Prevec, L., M. Schneider, et al. (1989). "Use of human adenovirus-based vectors for antigen expression in animals." J Gen Virol **70 ( Pt 2)**: 429-34.
- Regelson, W., S. Kuhar, et al. (1960). "Synthetic polyelectrolytes as tumour inhibitors." Nature **186**: 778-80.
- 5 Riviere, M., J. Tartaglia, et al. (1992). "Protection of mice and swine from pseudorabies virus conferred by vaccinia virus-based recombinants." J Virol **66(6)**: 3424-34.
- Robertson, E. S., T. Ooka, et al. (1996). "Epstein-Barr virus vectors for gene delivery to B lymphocytes." Proc Natl Acad Sci U S A **93(21)**: 11334-40.
- Robinson, H. L. and C. A. Torres (1997). "DNA vaccines." Semin Immunol **9(5)**: 271-83.
- 10 Roizman, B. (1996). "The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors." Proc Natl Acad Sci U S A **93(21)**: 11307-12.
- Sambrook, J. and D. W. Russell (2001). Molecular Cloning: a laboratory manual / Joseph Sambrook, David W. Russell. Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press.
- 15 Schneider, K., F. Puehler, et al. (2000). "cDNA cloning of biologically active chicken interleukin-18." J Interferon Cytokine Res **20(10)**: 879-83.
- Shida, H. (1986). "Nucleotide sequence of the vaccinia virus hemagglutinin gene." Virology **150(2)**: 451-62.
- Smith, G. E., M. D. Summers, et al. (1983). "Production of human beta interferon in insect cells infected with a baculovirus expression vector." Mol Cell Biol **3(12)**: 2156-65.
- 20 Snedecor, G. W. & COCHRAN, W. G. (1971) Transformation de proportions en Arcsinus. In Méthodes Statistiques. 6th edn. Eds H. Boelle, E. Camhaji. Association de Coordination Technique Agricole. pp 366-367
- Stickl, H. and V. Hochstein-Mintzel (1971). "[Intracutaneous smallpox vaccination with a weak pathogenic vaccinia virus ("MVA virus").]" Munch Med Wochenschr **113(35)**: 1149-53.
- 25 Stittelaar, K. J., L. S. Wyatt, et al. (2000). "Protective immunity in macaques vaccinated with a modified vaccinia virus Ankara-based measles virus vaccine in the presence of passively acquired antibodies." J Virol **74(9)**: 4236-43.
- 30 Sutter, G. and B. Moss (1992). "Nonreplicating vaccinia vector efficiently expresses recombinant genes." Proc Natl Acad Sci U S A **89(22)**: 10847-51.



- Sutter, G., L. S. Wyatt, et al. (1994). "A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to FMDV virus." Vaccine **12**(11): 1032-40.
- 5 Tang, D. C., M. DeVit, et al. (1992). "Genetic immunization is a simple method for eliciting an immune response." Nature **356**(6365): 152-4.
- Taylor, J., R. Weinberg, et al. (1988). "Protective immunity against avian FMDV induced by a fowlpox virus recombinant." Vaccine **6**(6): 504-8.
- 10 Thompson, J. D., D. G. Higgins, et al. (1994). "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." Nucleic Acids Res **22**(22): 4673-80.
- Ulmer, J. B., J. J. Donnelly, et al. (1993). "Heterologous protection against FMDV by injection of DNA encoding a viral protein." Science **259**(5102): 1745-9.
- 15 Van der Zee, R., W. Van Eden, et al. (1989). "Efficient mapping and characterization of a T cell epitope by the simultaneous synthesis of multiple peptides." Eur J Immunol **19**(1): 43-7.
- van Ooyen, A., J. van den Berg, et al. (1979). "Comparison of total sequence of a cloned rabbit beta-globin gene and its flanking regions with a homologous mouse sequence." Science **206**(4416): 337-44.
- 20 Vialard, J., M. Lalumiere, et al. (1990). "Synthesis of the membrane fusion and hemagglutinin proteins of measles virus, using a novel baculovirus vector containing the beta-galactosidase gene." J Virol **64**(1): 37-50.
- Xin, K. Q., K. Hamajima, et al. (1999). "IL-15 expression plasmid enhances cell-mediated immunity induced by an HIV-1 DNA vaccine." Vaccine **17**(7-8): 858-66.

25

## CLAIMS

What we claim is:

1. A composition comprising an FMDV antigen and a pharmaceutical or veterinarily acceptable carrier, excipient, or vehicle.

2. The composition of claim 1, wherein the FMDV antigen is selected from the group consisting of FMDV P1-3C, FMDV P1, FMDV VP0, FMDV VP1, FMDV VP3, FMDV VP2, and FMDV VP4.

3. The composition of claim 1 or 2, wherein the FMDV antigen is expressed in a plant or microalga.

4. The composition of any one of claims 1-3, wherein the FMDV antigen is partially purified.

5. The composition of any one of claims 1-3, wherein the FMDV antigen is substantially purified.

6. The composition of any one of claims 1-5, wherein the FMDV antigen has at least 80% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29.

7. The composition of any one of claims 1-6, wherein the FMDV antigen is encoded by a polynucleotide having at least 70% sequence identity to the sequence as set forth in SEQ ID NOs:1, 2, 4, 8, 9, 11, 13, 14, 15, 16, 18, 19, 21, 22, 24, 25, 27, 28, 30-35.

8. The composition of any one of claims 1-7, wherein the pharmaceutical or veterinarily acceptable carrier, excipient, or vehicle is a water-in-oil emulsion or an oil-in-water emulsion.

9. A method of vaccinating a host susceptible to ovine, bovine, caprine, or porcine FMDV comprising at least one administration of the composition according to any one of claims 1 to 8.

10. A method of vaccinating a host susceptible to ovine, bovine, caprine, or porcine FMDV comprising a prime-boost administration protocol.

11. The method of claim 10, wherein said prime-boost administration comprises a prime-administration of the composition of any one of claims 1-8, and a boost-administration

of a vaccine or composition comprising a recombinant viral vector that contains and expresses the FMDV antigen *in vivo*, or an inactivated viral vaccine comprising the FMDV antigen, or a DNA plasmid vaccine or composition that contains or expresses the FMDV antigen.

12. The method of claim 10, wherein the prime-boost administration comprises a prime-administration of a vaccine or composition comprising a recombinant viral vector that contains and expresses the FMDV antigen *in vivo*, or an inactivated viral vaccine comprising the FMDV, or a DNA plasmid vaccine or composition that contains or expresses the FMDV antigen, and a boost-administration of the composition of any one of claims 1-8.

13. The method of claim 10, wherein the prime-boost administration comprises a prime-administration of the composition of any one of claims 1-8, and a boost-administration of the composition of any one of claims 1-8.

14. The method of any one of claims 9-13, wherein the host is ovine, bovine, caprine, or porcine.

15. A substantially purified FMDV polypeptide expressed in a plant or microalga, wherein the polypeptide comprises:

a) an amino acid sequence having at least 80% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29;

b) a conservative variant of the amino acid sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29;

c) an immunogenic fragment comprising at least eight consecutive amino acids of the amino acid sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29, that specifically binds to an antibody that specifically binds to the amino acid sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29.

16. A plasmid comprising a DNA fragment having at least 70% sequence identity to the sequence as set forth in SEQ ID NO:1, 2, 4, 8, 9, 11, 13, 14, 15, 16, 18, 19, 21, 22, 24, 25, 27, 28, 30-35.

17. The plasmid of claim 16, wherein the plasmid is for plant transformation.

18. A host cell transformed with the plasmid of claim 17.

19. A stably transformed duckweed plant or microalga culture transformed with a gene expressing an FMDV antigen or fragment or variant thereof.

20. The duckweed plant or microalga culture of claim 19, wherein the antigen or fragment or variant thereof has at least 80% sequence identity to the sequence as set forth in SEQ ID NOs:3, 10, 12, 17, 20, 23, 26, or 29.

21. A method of producing an FMDV antigen comprising: (a) culturing within a duckweed culture medium a duckweed plant culture or a duckweed nodule culture, wherein the duckweed plant culture or the duckweed nodule culture is stably transformed to express the antigen, and wherein the antigen is expressed from a nucleotide sequence comprising a coding sequence for the antigen; and (b) collecting the antigen from the culture biomass.

FIG. 1

SEQ ID NO	TYPE	Description
1	DNA	pHM1119.1 FMDV P1-3C optimized for mammalian expression
2	DNA	FMDV – P1-3C (MerE01 – MerE04) optimized for Lemna expression
3	PRT	FMDV – P1-3C (MerE01 – MerE04) translation of SEQ ID NOs:1 and 2
4	DNA	Nucleotides 1222-1775 of <i>Zea mays</i> alcohol dehydrogenase 1 gene
5	PRT	<i>Arabidopsis thaliana</i> basic endochitinase signal peptide
6	PRT	Rice $\alpha$ -amylase signal sequence peptide (translation of SEQ ID NO:8)
7	PRT	Modified rice $\alpha$ -amylase signal sequence peptide
8	DNA	Rice $\alpha$ -amylase signal sequence nucleic acid
9	DNA	FMDV – P1 DNA optimized for Lemna expression
10	PRT	FMDV – P1 protein (translation of SEQ ID NOs:9 and 13)
11	DNA	FMDV – 3C DNA optimized for Lemna expression
12	PRT	FMDV – 3C protein (translation of SEQ ID NOs:11 and 14)
13	DNA	FMDV – P1 DNA optimized for mammalian expression
14	DNA	FMDV – 3C DNA optimized for mammalian expression
15	DNA	FMDV – VP0 DNA optimized for Lemna expression
16	DNA	FMDV – VP0 DNA optimized for mammalian expression
17	PRT	FMDV – VP0 protein (translation of SEQ ID NOs:15 and 16)
18	DNA	FMDV – VP3 DNA optimized for Lemna expression
19	DNA	FMDV – VP3 DNA optimized for mammalian expression
20	PRT	FMDV – VP3 protein (translation of SEQ ID NOs:18 and 19)
21	DNA	FMDV – VP1 DNA optimized for Lemna expression
22	DNA	FMDV – VP1 DNA optimized for mammalian expression
23	PRT	FMDV – VP1 protein (translation of SEQ ID NOs:21 and 22)
24	DNA	FMDV – VP2 DNA optimized for Lemna expression
25	DNA	FMDV – VP2 DNA optimized for mammalian expression
26	PRT	FMDV – VP2 protein (translation of SEQ ID NOs:24 and 25)
27	DNA	FMDV – VP4 DNA optimized for Lemna expression
28	DNA	FMDV – VP4 DNA optimized for mammalian expression
29	PRT	FMDV – VP4 protein (translation of SEQ ID NOs:27 and 28)
30	DNA	MERF01 plasmid
31	DNA	MERF02 plasmid
32	DNA	MERF03 plasmid
33	DNA	MERF04 plasmid
34	DNA	MERF05 plasmid
35	DNA	MERF06 plasmid
36	DNA	MERE01 plasmid
37	DNA	MERE02 plasmid
38	DNA	MERE03 plasmid
39	DNA	MERE04 plasmid

FIG. 2

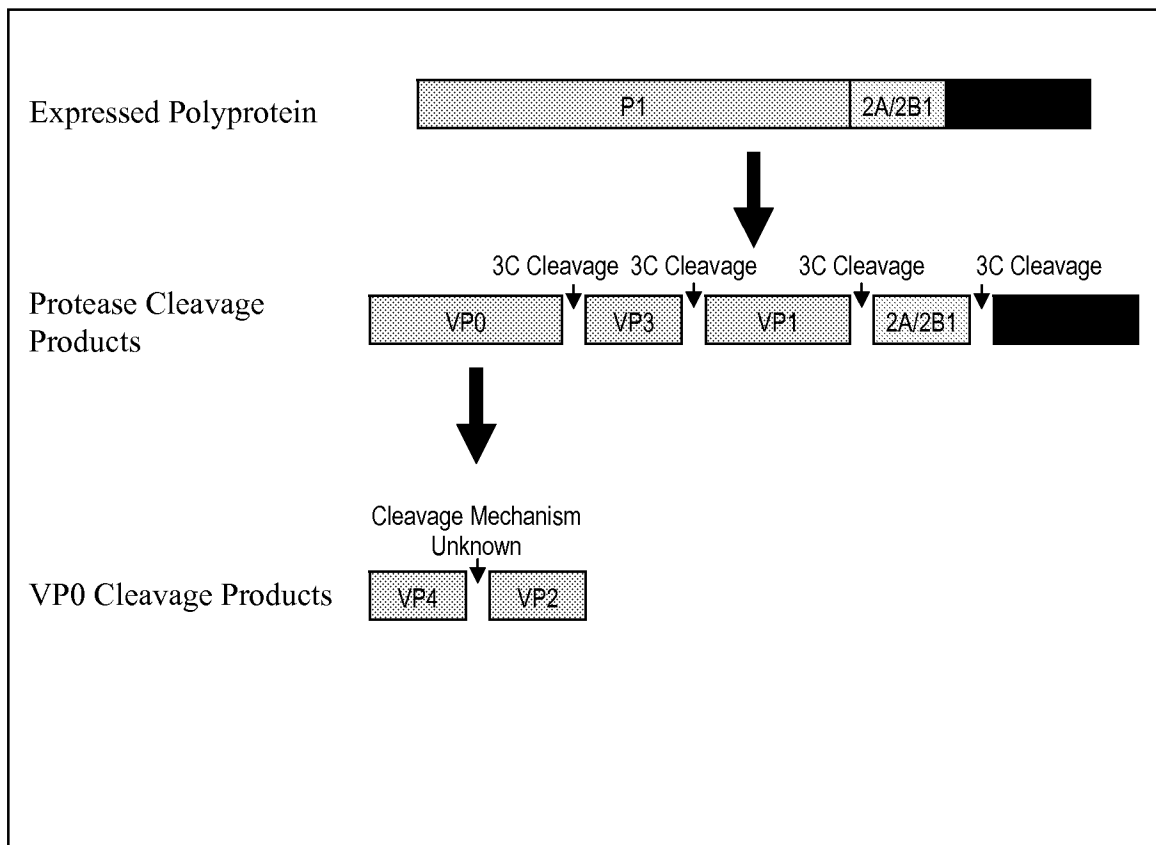
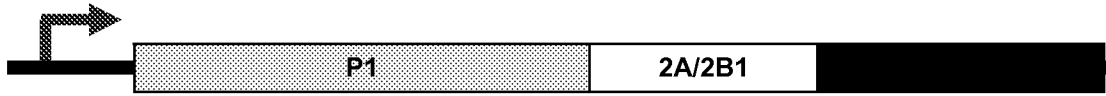


FIG. 3

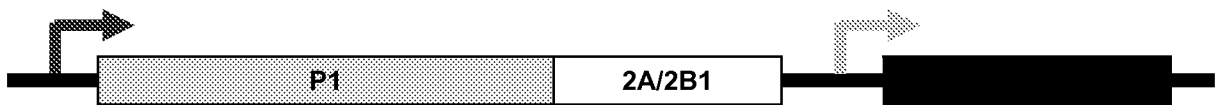
**MerE01: Strong Promoter + P1+2A/2B1 (A24) + 3C (A12)**



**MerE02: Strong Promoter + P1**



**MerE03: Strong Promoter + P1+2A/2B1 (A24) + Weak Promoter 3C (A12)**



**MerE04: Strong Promoter + P1+2A-2B1 (A24) + Weak Promoter 3C (A12)  
with optimized 5'UTR**

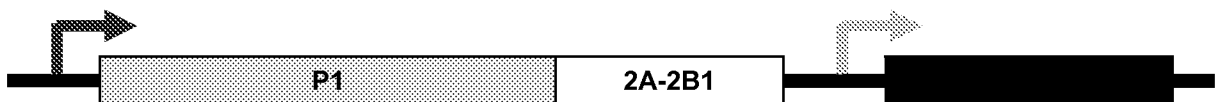


FIG. 4 (1/4)

		1	50
FMDV Opti.	(1)	ATGGGCGCCGGGCAGTCCCTCGCCTGCGACGGGGAGCCAGAACCAGTCGGG	
FMDV Orig.	(1)	ATGGGAGCTGGGCAATCCAGCCAGCAACCGGCTCGCAGAACCAGTCTGG	
		51	100
FMDV Opti.	(51)	CAACACCGGCTCGATCATCAACAACACTACTACATGCAGCAATACCAGAACA	
FMDV Orig.	(51)	CAACACTGGCAGCATAATCAACAACACTACTACATGCAACAGTACCAGAACT	
		101	150
FMDV Opti.	(101)	GCATGGACACCCAGCTCGGCGATAACGCCATCTCCGGCGGGTCCAACGGAG	
FMDV Orig.	(101)	CCATGGACACACAGTTGGGAGACAATGCCATCAGTGGAGGCTCCAACGGAG	
		151	200
FMDV Opti.	(151)	GGCTCGACCGACACGACCTCCACCCACACCACGAACACCCAGAACAATGA	
FMDV Orig.	(151)	GGCTCCACGGACACAACCTTCAACACACACAACCAACACTCAAAACAATGA	
		201	250
FMDV Opti.	(201)	CTGGTTCAGCAAGCTGGCCTCCAGCGCCTTCACGGGCCTGTTCCGGGGCGC	
FMDV Orig.	(201)	CTGGTTCCTCGAAGCTCGCCAGTTCAGCTTTTACCGGTCTGTTCCGGTGCAC	
		251	300
FMDV Opti.	(251)	TGCTCGCGGACAAGAAGACCGAGGAGACCACGCTGCTCGAGGACCCGGATC	
FMDV Orig.	(251)	TGCTCGCCGACAAGAAGACAGAGGAAACGACACTTCTTGAGGACCCGCATC	
		301	350
FMDV Opti.	(301)	CTCACCACGCGCAACGGGCACACGACCAGCAGCAGCCAGTCCAGCGTCCG	
FMDV Orig.	(301)	CTCACCACCCGCAACGGGCACACCACCTCGACGACCCAATCGAGTGTGGG	
		351	400
FMDV Opti.	(351)	GGTGACCCACGGCTACTCCACGGAGGAGGACCACGTCGCCGGGCCGAACA	
FMDV Orig.	(351)	TGTCACACACGGGTACTCCACAGAGGAGGACCACGTTGCTGGGCCAACA	
		401	450
FMDV Opti.	(401)	CGAGCGGCCTCGAGACCCGCGTGGTCCAGGGCGGAGCGCTTCTACAAGAAG	
FMDV Orig.	(401)	CATCGGGCCTGGAGACCGGAGTGGTGCAGGCAGAGAGATTCTACAAAAG	
		451	500
FMDV Opti.	(451)	TACCTGTTTCGACTGGACCACGGACAAGGCGTTCCGGGCACCTCGAGAAGCT	
FMDV Orig.	(451)	TACTTGTGTTGACTGGACAACGGACAAGGCATTTGGACACCTGGAAAGCT	
		501	550
FMDV Opti.	(501)	GGAGCTGCCGAGCGACCACCACGGCGTCTTCGGCCACCTCGTGGACTCCT	
FMDV Orig.	(501)	GGAGCTCCCGTCCGACCACCACGGTGTCTTTGGACACTTGGTGGACTCGT	
		551	600
FMDV Opti.	(551)	ACGCGTACATGAGAAATGGGTGGGACGTCGAGGTGAGCGCCGTCGGCAAC	
FMDV Orig.	(551)	ACGCCTATATGAGAAATGGCTGGGATGTTGAGGTGTCCGCTGTTGGCAAC	
		601	650
FMDV Opti.	(601)	CAGTTCAACGGCGGGTGCCTGCTCGTGGCGATGGTCCCAGGAGTGGAAAGGA	
FMDV Orig.	(601)	CAGTTCAACGGCGGGTGCCTCCTGGTGGCCATGGTACCTGAATGGAAAGGA	
		651	700
FMDV Opti.	(651)	GTTTCGACACCCGCGAGAAGTATCAGCTGACCCTCTTCCCCACCAGTTCA	
FMDV Orig.	(651)	ATTTGACACACGGGAGAAATACCAACTCACCCCTTTCCCCGACCAGTTTA	
		701	750
FMDV Opti.	(701)	TCAGCCCGCGCACCAACATGACCGCCACATCACGGTGCCGTACCTCGGG	
FMDV Orig.	(701)	TTAGCCCCAGAACTAACATGACTGCCACATCACGGTCCCCCTACCTGGT	
		751	800
FMDV Opti.	(751)	GTGAACCGCTACGACCAGTACAAGAAGCACAAGCCCTGGACCTTCGTGGT	
FMDV Orig.	(751)	GTGAACAGGTATGATCAGTACAAGAAGCATAAGCCCTGGACATTTGGTTGT	
		801	850
FMDV Opti.	(801)	CATGGTGGTCTCCCCGCTGACTGTGAACAACACGTCCGCCGGGCAGATCA	
FMDV Orig.	(801)	CATGGTCTGTGCGCACTTACGGTCAACAACACTAGTGGCGGCACAAATCA	
		851	900
FMDV Opti.	(851)	AAGTCTACGCCAACATCGCCCGCACCTACGTGCACGTCCGCCGGCGAGCTG	
FMDV Orig.	(851)	AGGTCTACGCCAACATAGCTCCGACCTATGTTTACCGTGGCCGGTGAAGTC	



FIG. 4 (2/4)

		901		950
FMDV Opti.	(901)	CCCTCCAAGGAGGGCATCTTCCCGCTCGCCTGCGCCGACGGGTACGGGGG		
FMDV Orig.	(901)	CCCTCGAAAGAGGGGATTTTCCCGGTTGCATGTGCGGACGGTTACGGAGG		
		951		1000
FMDV Opti.	(951)	CCTGGTGACCACCGACCCCAAGACGGCCGACCCGGCCTACGGCAAAGTGT		
FMDV Orig.	(951)	ATTGGTGACGACAGACCCGAAGACAGCTGACCCCTGCTTATGGCAAGGTGT		
		1001		1050
FMDV Opti.	(1001)	ACAACCCGCCCAGGACGAACCTACCCCGGTTCGCTTCACCAACCTCCTGGAC		
FMDV Orig.	(1001)	ACAACCCGCTTAGGACTAACTACCCCTGGGCGCTTCACCAACCTGTTGGAC		
		1051		1100
FMDV Opti.	(1051)	GTGGCGGAGGCCTGCCCGACCTTCCTGTGCTTCGACGACGGGAAGCCCTA		
FMDV Orig.	(1051)	GTGGCCGAAGCGTGTCCACTTTCCCTCTGCTTTGACGACGGGAACCGTA		
		1101		1150
FMDV Opti.	(1101)	CGTCACCACGCGCACCCGACGACAGCGCCTGCTCGCCAAGTTCGACCTCA		
FMDV Orig.	(1101)	CGTCACCACGCGGACGGATGACACCCGACTTTTGGCCAAGTTCGACCTTT		
		1151		1200
FMDV Opti.	(1151)	GCCITGGCCGCTAAGCACATGAGCAACACCTACCTCAGCGGCATCGCCCA		
FMDV Orig.	(1151)	CCCTTGGCCGCAAAACATATGTCCAACACATACCTGTGAGGGATGCTCAG		
		1201		1250
FMDV Opti.	(1201)	TACTATACCCAATACTCGGGCACCATCAACCTGCACCTTCATGTTACGGG		
FMDV Orig.	(1201)	TACTACACACAGTACTCTGGCACCATCAATTTGCATTTTCATGTTAGAGG		
		1251		1300
FMDV Opti.	(1251)	CAGCACCGACAGCAAGGCCCGGTACATGGTGGCCTACATCCCGCCGGGCG		
FMDV Orig.	(1251)	TTCCACTGATTCAAAGGCCCGATACATGGTGGCCTACATCCCACCTGGGG		
		1301		1350
FMDV Opti.	(1301)	TGGAGACCCCTCCCGACACGCCGAGCGGGCTGGCGACTGCATCCACGGC		
FMDV Orig.	(1301)	TGGAGACACCACCGGACACACCTGAAAGGGCTGCCCACTGCATTCACGGT		
		1351		1400
FMDV Opti.	(1351)	GAGTGGGACACCGGCCTCAACAGCAAGTTCACGTTTCAGCATCCCCACCGT		
FMDV Orig.	(1351)	GAATGGGACACTGGACTAAACTCCAATTCACCTTCTCAATCCCGTACGT		
		1401		1450
FMDV Opti.	(1401)	GTCCGCCGCGGATTACGCTTACACCGCCTCGGACACGGCCGAGACGATCA		
FMDV Orig.	(1401)	ATCCGCCGCGGATTACGCGTACACAGCGTCTGACACGGCAGAAACAATCA		
		1451		1500
FMDV Opti.	(1451)	ACGTCCAGGGCTGGGTCTGCATCTACCAGATCACTCACGGCAAGGCCGAG		
FMDV Orig.	(1451)	ACGTACAGGGATGGGTCTGCATCTACCAATTACACACGGGAAGGCTGAA		
		1501		1550
FMDV Opti.	(1501)	AACGACACCCTCGTCGTGAGCGTCTCCGCCGGGAAGGACTTCGAGCTGAG		
FMDV Orig.	(1501)	AATGACACCTTGGTCGTGTCGTTAGCGCCGGCAAAGACTTTGAGTTGCG		
		1551		1600
FMDV Opti.	(1551)	GCTGCCCATCGACCCCAAGCAGCAGACCACGGCGACCGGGGAGTCCGCCG		
FMDV Orig.	(1551)	CCTCCCGATTGACCCCCGCCAGCAGACCACCGCTACCGGGGAATCAGCAG		
		1601		1650
FMDV Opti.	(1601)	ACCCCGTGACCACGACCGTGGAGAACTACGGCGGGGAGACTCAGATCCAG		
FMDV Orig.	(1601)	ACCCGGTACCACCACCGTGGAGAACTACGGCGGTGAGACACAAATCCAG		
		1651		1700
FMDV Opti.	(1651)	CGGCCACCACACCGACATCGGCTTCATCATGGACCGCTTCGTGAAGAT		
FMDV Orig.	(1651)	AGACGTCACCACACGGACATTGGTTTCATCATGGACAGATTTGTGAAGAT		
		1701		1750
FMDV Opti.	(1701)	CCAGTCCCTGTGCGCCACCCACGTTATCGACCTCATGCAGGCCACCAGC		
FMDV Orig.	(1701)	CCAAAGCTTGAGCCCAACACATGTCATTTGACCTCATGCAGGCTCACCAAC		
		1751		1800
FMDV Opti.	(1751)	ACGGGCTCGTGGGTGCCCTCCTGCGCGCGGCCACCTACTACTTCAGCGAC		
FMDV Orig.	(1751)	ACGGTCTGGTGGGTGCCCTTGCTGCGTGCAGCCACGTAATACTTTCTGAC		

FIG. 4 (3/4)

		1801		1850
FMDV Opti.	(1801)	CTCGAGATCGTTGTCCGCCACGAGGGGAACTCACCTGGGTCCCGAACGG		
FMDV Orig.	(1801)	CTGGAAATTGTTGTACGGCACGAAGGCAATCTGACCTGGGTGCCCAACGG		
		1851		1900
FMDV Opti.	(1851)	TGCCCCGAGAGCGCCCTGCTCAACACCTCCAACCCCACGGCGTACAACA		
FMDV Orig.	(1851)	CGCCCCGAAATCAGCCCTGTTGAACACCAGCAACCCCCTGCTACAACA		
		1901		1950
FMDV Opti.	(1901)	AGGCCCCCTTCACGCGCCTCGCCCTGCCTTACACCGCGCCCCACCGCGTG		
FMDV Orig.	(1901)	AGGCACCAATTCACGAGACTCGCTCTCCCCTACACTGCGCCGCACCGTGTG		
		1951		2000
FMDV Opti.	(1951)	CTGGCCACGGTGTACAACGGGACCTCCAAGTACGCCCTGGGCGGGAGCGG		
FMDV Orig.	(1951)	CTGGCAACAGTGTACAACGGGACGAGTAAGTATGCTGTGGGTGGTTCAGG		
		2001		2050
FMDV Opti.	(2001)	CCGCAGAGGGCGACATGGGGAGCCTCGCCCTAGGGTCTGTGAAGCAGCTCC		
FMDV Orig.	(2001)	CAGAAGAGGGCGACATGGGGTCTCTCGCGCGCGAGTCTGTGAACAGCTTC		
		2051		2100
FMDV Opti.	(2051)	CGGCCTCCTTCAACTACGGCGCCATCAAGGCCGACGCCATCCACGAACTC		
FMDV Orig.	(2051)	CTGCTTCATTTAACTACGGTGAATCAAGGCCGACGCCATCCACGAACTT		
		2101		2150
FMDV Opti.	(2101)	CTGGTCCGCATGAAGCGCGCCGAACTCTACTGCCCCCGCCCTCCTGGC		
FMDV Orig.	(2101)	CTCGTGCCATGAAACGGCCGAGCTCTACTGCCCCAGACCGCTGTGGC		
		2151		2200
FMDV Opti.	(2151)	CATCGAGGTCTCCTCGCAGGACCGCCACAAGCAGAAGATCATCGCCCGG		
FMDV Orig.	(2151)	ARTAGAGGTGTCTTCGCAAGACAGGCACAAGCAAAAGATCATTCACCAG		
		2201		2250
FMDV Opti.	(2201)	CCAAGCAGCTGCTCAACTTCGACCTGCTCAAGCTCCCGGGGACGTCGAG		
FMDV Orig.	(2201)	CAAAGCAGCTTCTGAATTTTGCACCTGCTCAAGTTGGCCGGAGACGTTGAG		
		2251		2300
FMDV Opti.	(2251)	TCAACCCCGGTCCCTTCTTCTTCGCCGACGTCGGTCCAAGTCTCGAA		
FMDV Orig.	(2251)	TCAACCCCGGGCCATTCTTCTTCTGCTGACGTTAGGTCAAAGTCTTCAA		
		2301		2350
FMDV Opti.	(2301)	GCTCGTGGACACCATCAACCAGATGCAGGAGGACATGAGCACCAAGCAG		
FMDV Orig.	(2301)	GTTGGTAGACACAATCAACCAGATGCAGGAGGACATGTCCACAAAACAG		
		2351		2400
FMDV Opti.	(2351)	GGCCGGACTTCAACCGCCTCGTCTCGGCCCTTCGAGGAACTCGCCACCGG		
FMDV Orig.	(2351)	GGCCCGACTTCAACCGGTTGGTGTCCGCAATTCGAGGAAATGGCCACTGGA		
		2401		2450
FMDV Opti.	(2401)	GTC AAGGCCATCAGGACGGGCTGGACGAGGCCAAGCCGTGGTACAAGCT		
FMDV Orig.	(2401)	GTTAAGCTATCAGGACCGGTCTCGACGAGGCCAAGCCGTGGTACAAGCT		
		2451		2500
FMDV Opti.	(2451)	CATCAAGCTCCTGTTCGGCCTCTCCTGCATGGCGGCTGTTCGGGCCCGCT		
FMDV Orig.	(2451)	TATCAAATCCTAAGCCGCCTGTCTGTGCATGGCCGCTGTGGCAGCACGGT		
		2501		2550
FMDV Opti.	(2501)	CCAAGGACCCCGTCTCTCGTCGCCATCATGCTCGCGGACACCCGGCTCGAG		
FMDV Orig.	(2501)	CCAAGGACCCAGTCTCTTGTGGCCATCATGCTGGCCGACACCCGGTCTCGAG		
		2551		2600
FMDV Opti.	(2551)	CGCCAGCGGCCCTGAAAGTGC CGCC AAGCTGCCGCAGCAGGAGGGCCC		
FMDV Orig.	(2551)	CGTCAGAGACCTCTGAAAGTGAGAGCTAAGCTCCCACAGCAGGAAGGACC		
		2601		2650
FMDV Opti.	(2601)	CTACGCCGGCCCGCTCGAGAGACAGAAGCCGCTGAAAGTCAAGGCCAAGG		
FMDV Orig.	(2601)	TTACGCTGGCCCGTGTGGAGAGACAGAAACCCGCTGAAAGTGAAGCCAAAG		
		2651		2700
FMDV Opti.	(2651)	CGCCCGTGGTCAAGGAGGGGCCCTACGAGGGCCCCGTCAAGAAGCCGGTG		
FMDV Orig.	(2651)	CCCCGGTCTCAAGGAAGGACCTTACGAGGGACCGGTGAAGAAGCCGTG		

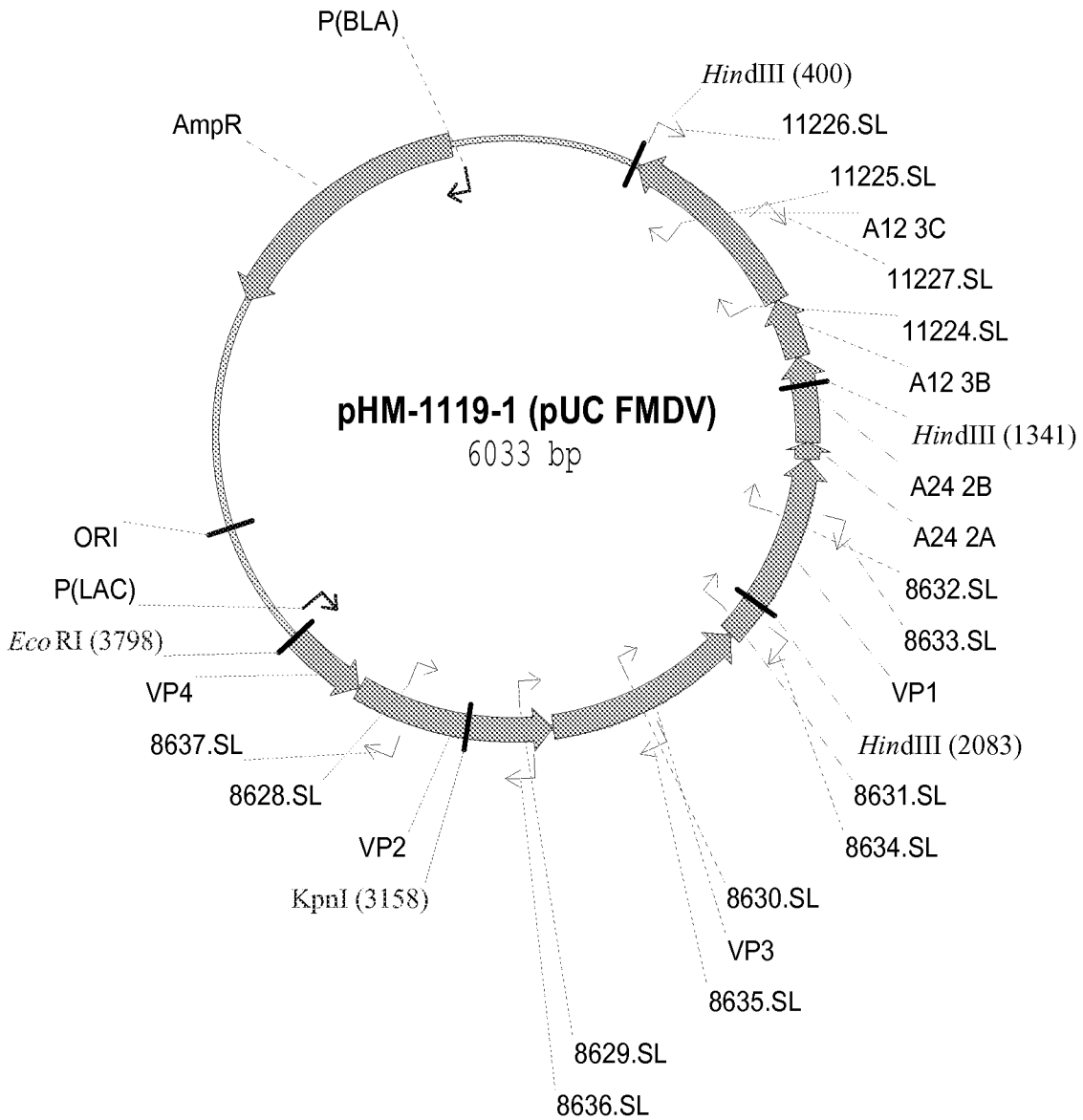
FIG. 4 (4/4)

```

2701                                     2750
FMDV Opti. (2701) GCGCTGAAAGTGAAGGCCAAGAACCTCATCGTGACCGAGTCCGGTGGGCC
FMDV Orig. (2701) GCTTTGAAAGTGAAGGCTAAGAACTTGATAGTCACTGAGAGTGGTGGCCC
2751                                     2800
FMDV Opti. (2751) TCCACCGACCTGCAGAAGATGGTGTATGGGCAACACCAAGCCCGTCCGAGT
FMDV Orig. (2751) ACCGACCGACTTGCAAAGATGGTCAATGGGCAACACAAAGCCTGTTGAGC
2801                                     2850
FMDV Opti. (2801) TGATCCTCGACGGGAAGACCGTGGCGAICTGCTGCGCCACCGGCGTGTTC
FMDV Orig. (2801) TCATCCTTGACGGGAAGACAGTAGCCATCTGTGTGTGCTACTGGAGTGTTC
2851                                     2900
FMDV Opti. (2851) GGCACCGCCTACCTCGTCCCAGACACCTGTTCCGCCGAGAAGTATGACAA
FMDV Orig. (2851) GGCACCGCCTACCTCGTCCCAGACACCTGTTCCGCCGAGAAGTATGACAA
2901                                     2950
FMDV Opti. (2901) GATCATGCTGGACGGGCGGGCCATGACCGACTCGGACTACCGGGTCTTCG
FMDV Orig. (2901) GATCATGCTGGATGGCAGAGCCATGACAGACAGTACTACAGAGTGTTCG
2951                                     3000
FMDV Opti. (2951) AGTTCGAGATCAAAGTGAAGGGCCAGGATATGCTCTCCGACGCCCGCGCTG
FMDV Orig. (2951) AGTTTGAGATTAAAGTAAAAGGACAGGACATGCTCTCAGACGCTGCGCTC
3001                                     3050
FMDV Opti. (3001) ATGGTGCTCCACAGAGGCCAACC CGGTGCGGGACATCACCAAGCACTTCAG
FMDV Orig. (3001) ATGGTGCTCCACCGTGGGAACCCGCGTGAGAGATATCAGGAAACACTTTCG
3051                                     3100
FMDV Opti. (3051) GGACACCGCGCGCATGAAGAAGGGGACCCCTGTGGTCCGGGGTCCGTGAACA
FMDV Orig. (3051) TGATACAGCAAGAATGAAGAAAGGCACCCCGTCCGTCGGTGTGGTCAACA
3101                                     3150
FMDV Opti. (3101) ACGCCGACGTCGGGCGCCTCATCTTCTCCGGCGAGGCCGCTGACCTACAAG
FMDV Orig. (3101) ACGCCGACGTTGGGAGACTGATTTTCTCTGGTGGAGGCCCTCACCTACAAG
3151                                     3200
FMDV Opti. (3151) GACATCGTCGTGTGCATGGACGGGGACACGATGCCGGGGCTCTTCGCCTA
FMDV Orig. (3151) GATATTGTAGTGTGCATGGACGGGAGACACCATGCCTGGCCTCTTTGCCTA
3201                                     3250
FMDV Opti. (3201) CAAGGCCGCGACCAAGGCCGGGTACTGCGGGGGTGGGGTGTCCGCCAAGG
FMDV Orig. (3201) CAAGGCCGCCACCAAGGCCAGGCTACTGTGGAGGAGCCGTTCTCGCCAAGG
3251                                     3300
FMDV Opti. (3251) ACGGCGCCGACACGTTTCATCGTGGGCACCCACTCCGCGCGGCGGTAAACGGC
FMDV Orig. (3251) ACGGGGCCGACACTTTTCATCGTCCGCACTCACTCCGCAGGAGGCAATGGA
3301                                     3350
FMDV Opti. (3301) GTCGGCTACTGCTCCTGCGTGTCCCGGTCCATGCTGCTGCGGATGAAGGC
FMDV Orig. (3301) GTTGGATACTGCTCATGCGTTTCCAGGTCCATGCTTCTCAGAATGAAGGC
3351                                     3378
FMDV Opti. (3351) CCACGTTGACCCCGAGCCGACGACGAG
FMDV Orig. (3351) ACACGTTGACCCTGAACCACAACACGAG
    
```

FMDV Opti. FMDV Orig.	FMDV Opti.	FMDV Orig.  80
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FIG. 5



A12 3C	Start: 413	End: 1051	(Complementary)
A12 3B1	Start: 1052	End: 1246	(Complementary)
A24 2B	Start: 1247	End: 1528	(Complementary)
A24 2A	Start: 1529	End: 1582	(Complementary)
VP1	Start: 1583	End: 2215	(Complementary)
VP3	Start: 2216	End: 2878	(Complementary)
VP2	Start: 2879	End: 3532	(Complementary)
VP4	Start: 3533	End: 3790	(Complementary)
AmpR	Start: 4976	End: 5833	(Complementary)
ORI	Start: 4214	End: 4214	

FIG. 6

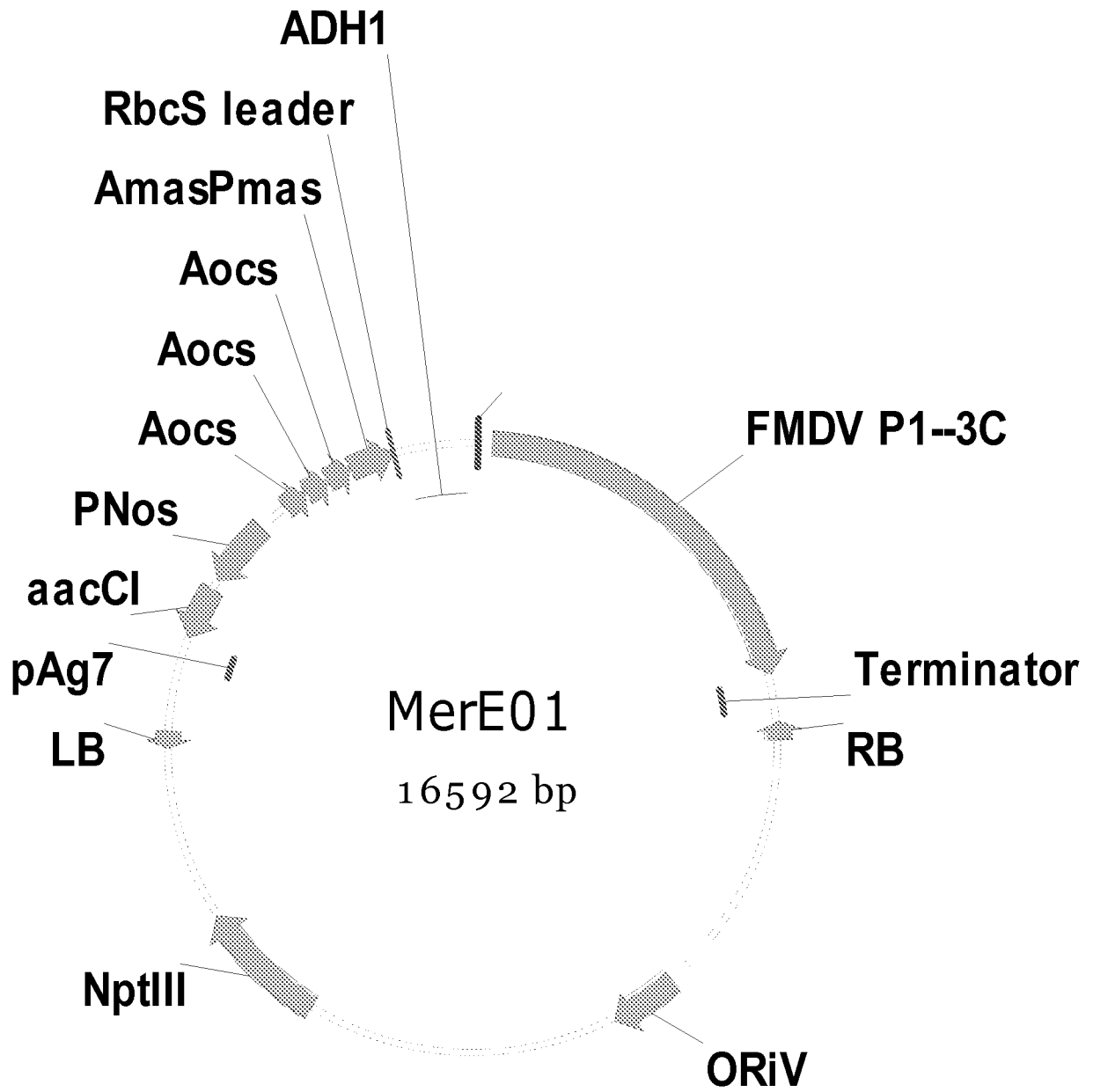


FIG. 7

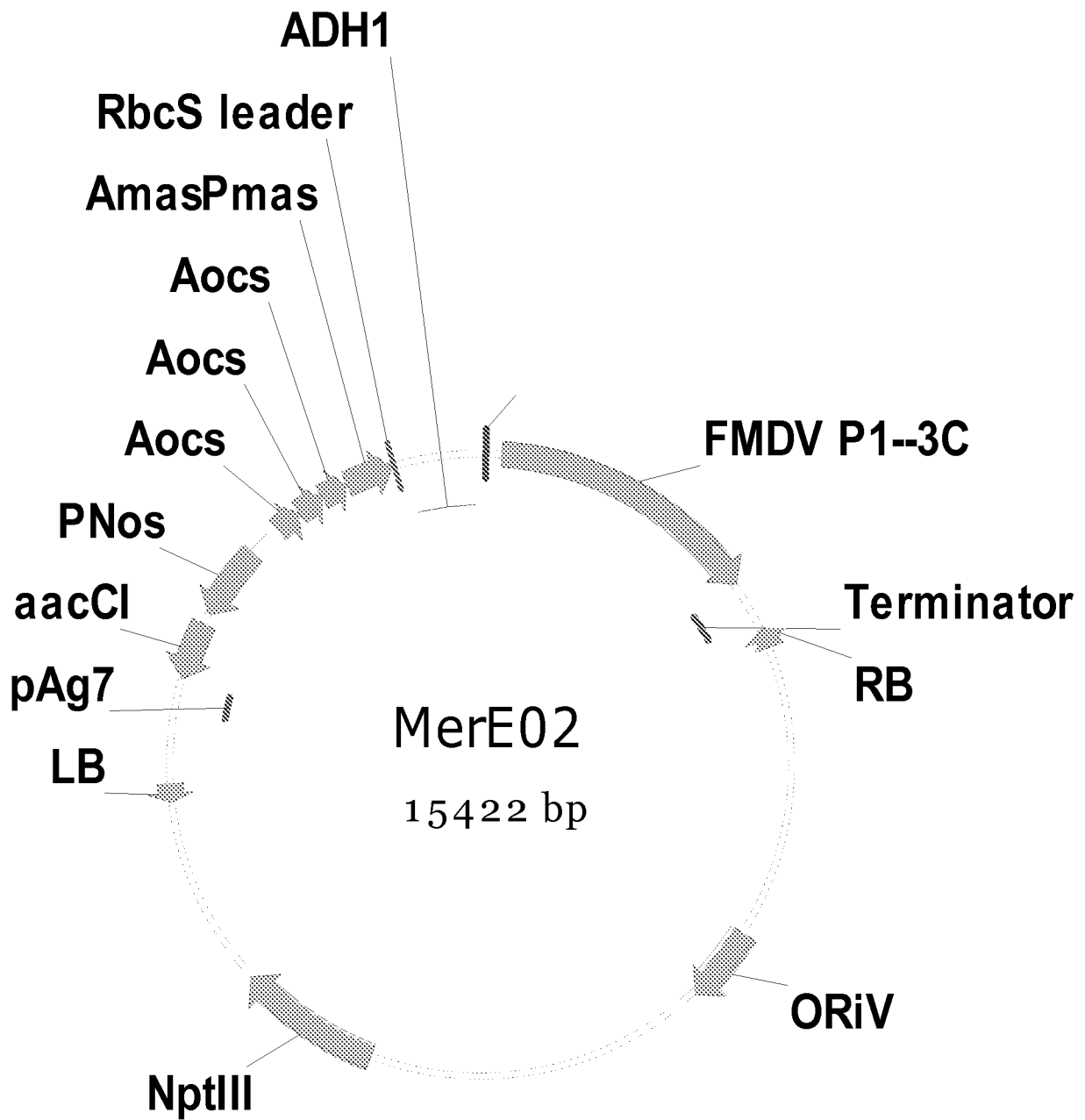


FIG. 8

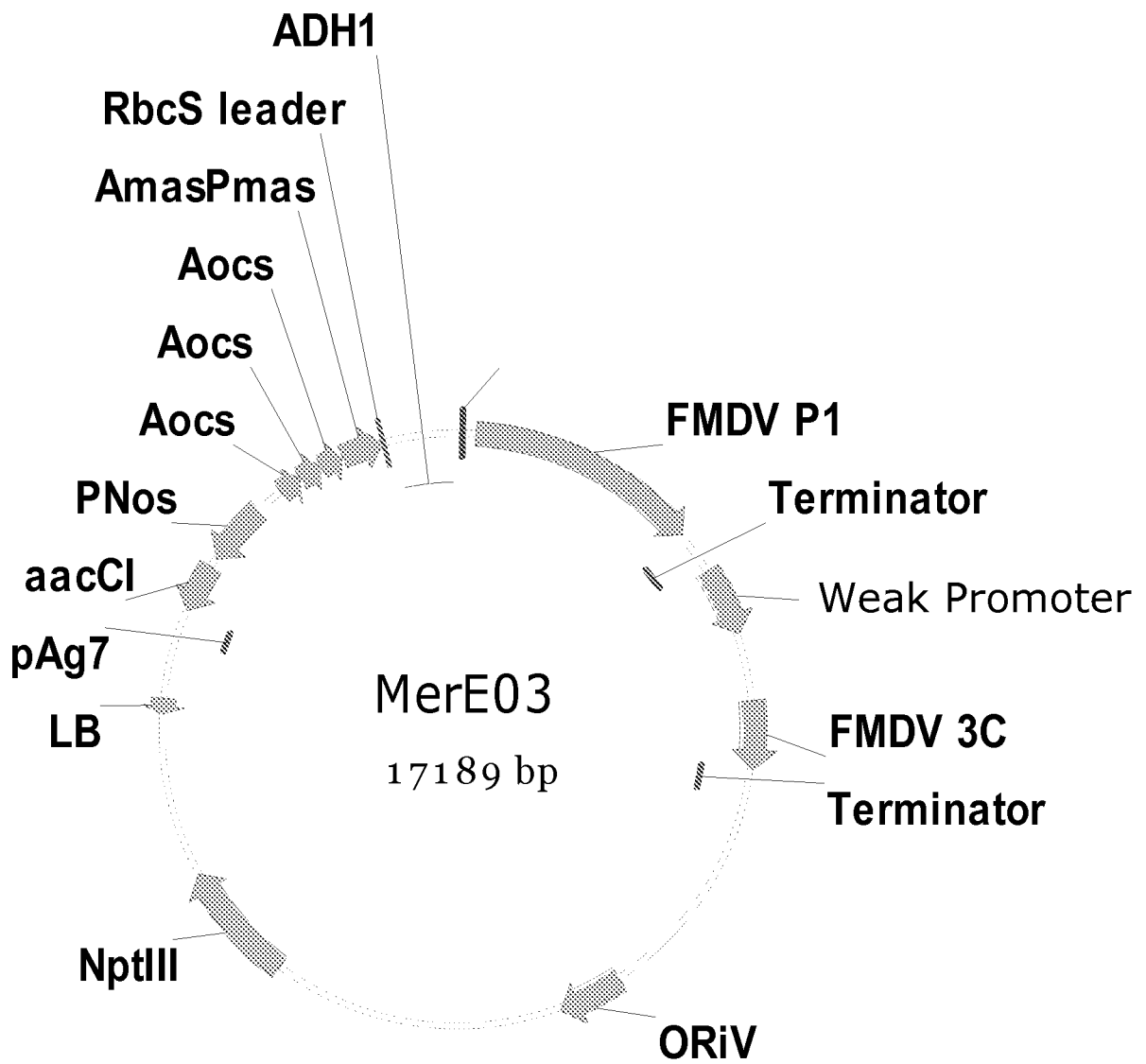


FIG. 9

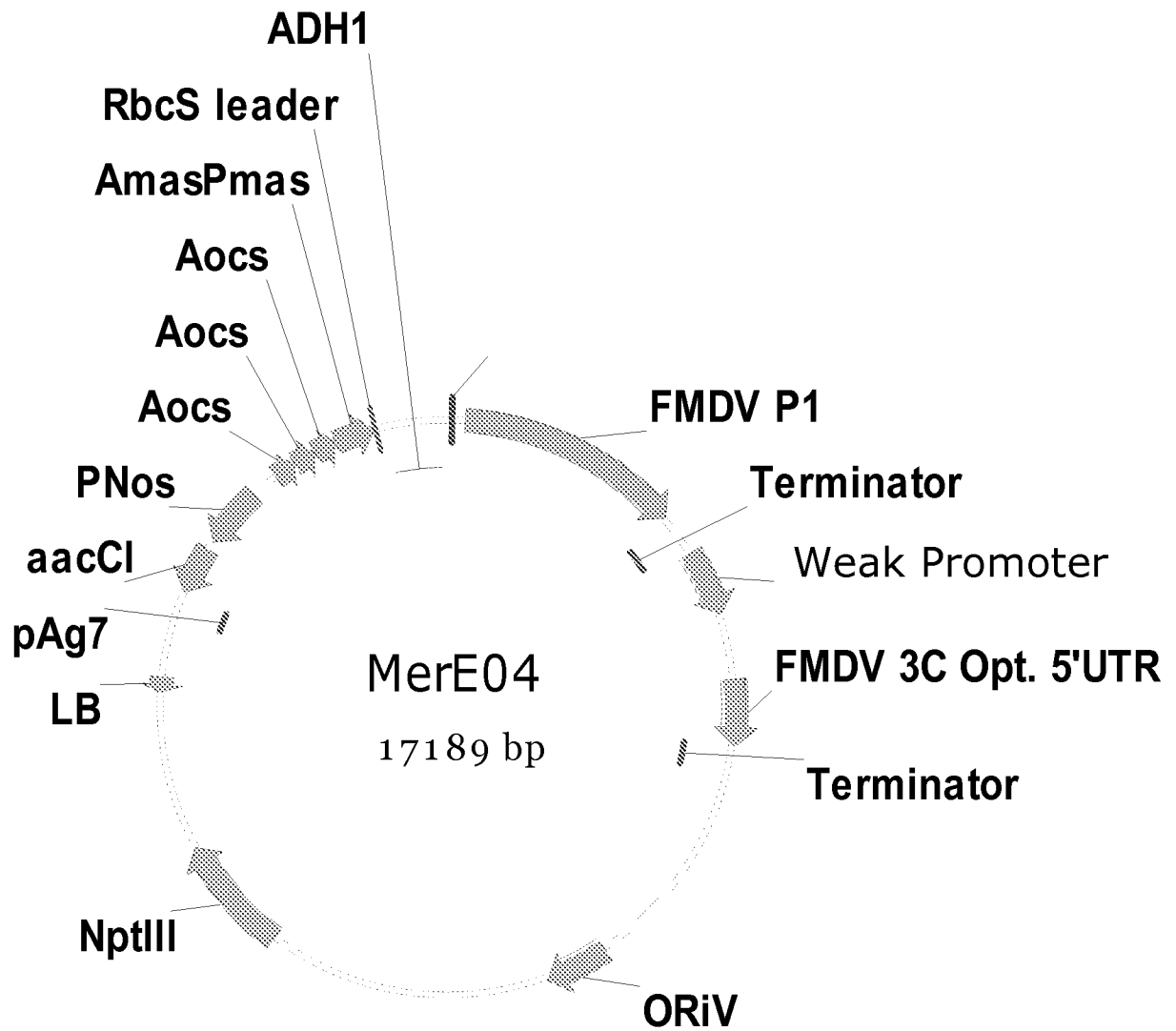
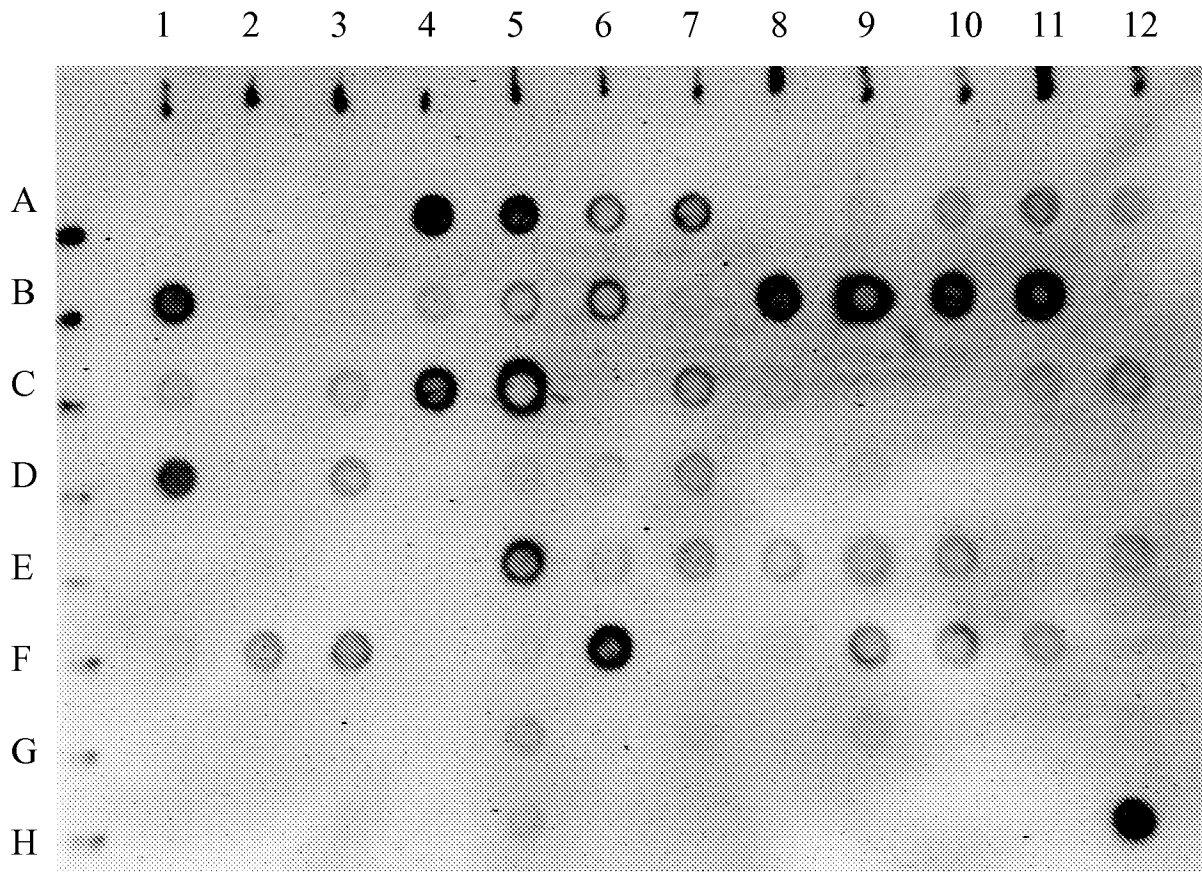




FIG. 10



RNA Dot Blot Probed with FMDV P1-specific probe.

FIG. 11

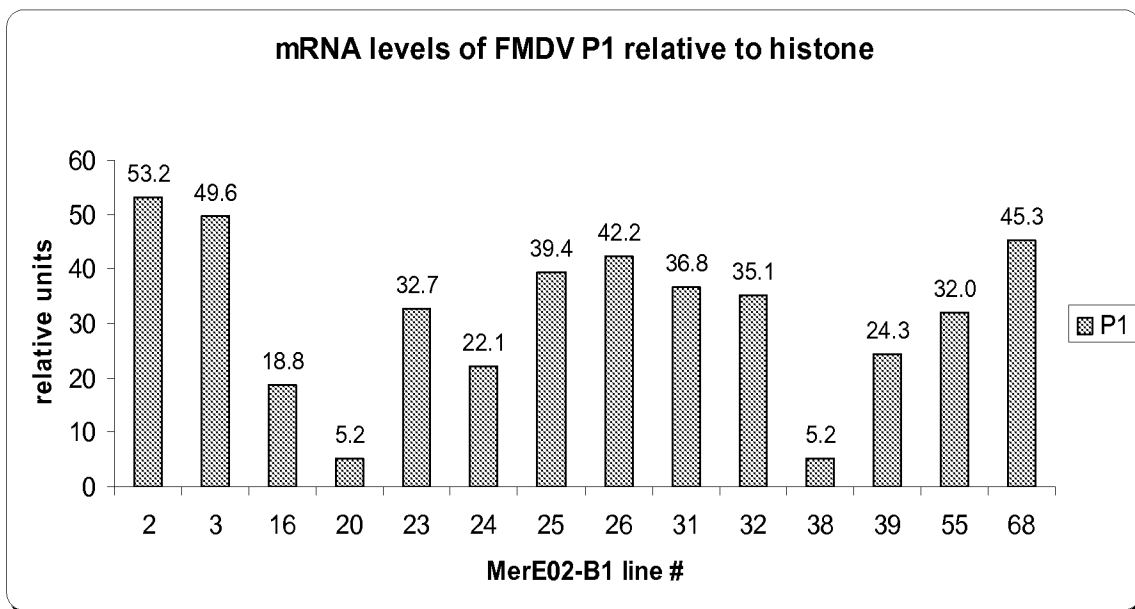
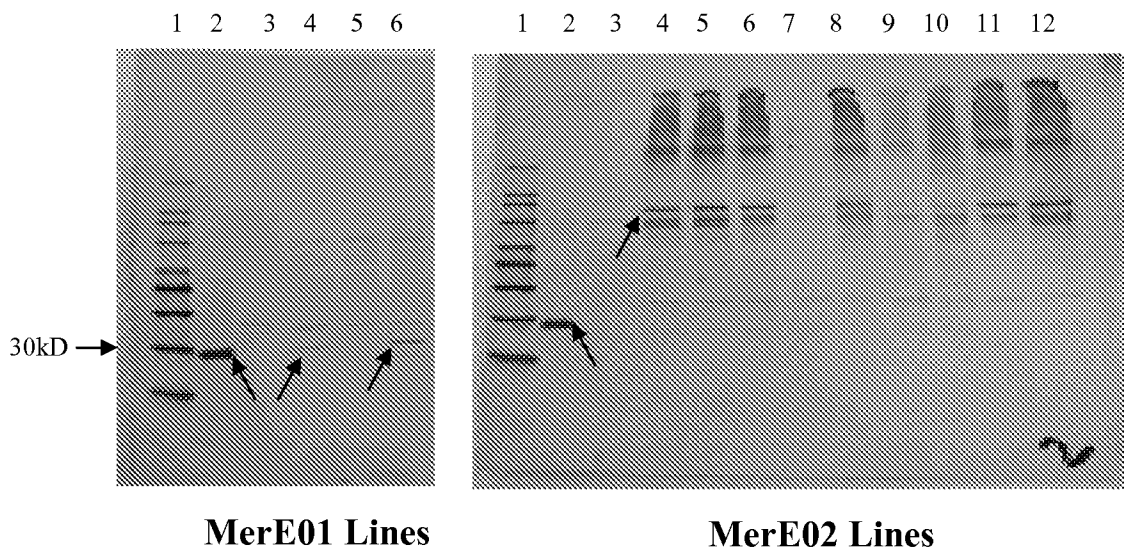


FIG. 12



**Arrows indicate FMDV bands.**

**Lanes:**

1 (both gels)=Magic Mark molecular weight marker

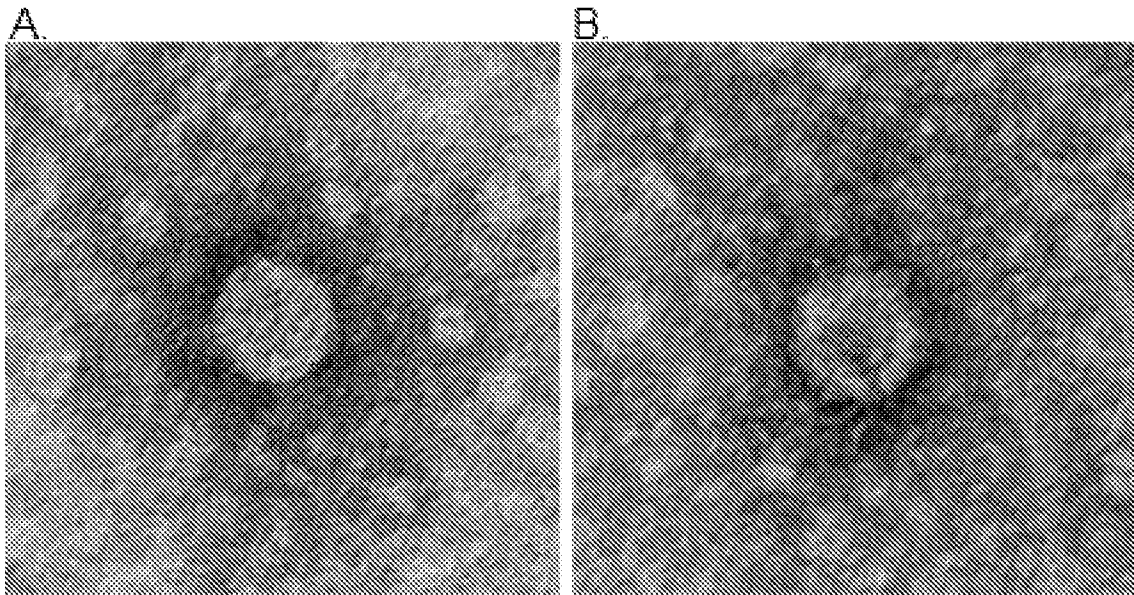
2 (both gels)=Purified FMDV A24 inactivated virus

3 (both gels)=Lemna wild type

4-6 of MerE01 Lines gel (left) are three lines - 6, 8 and 10

4-12 of MerE02 Lines gel (right) are nine lines - 2, 3, 16, 20, 23, 24, 25, 26 and 31

FIG. 13



100 nm

HV=120.0kV

Direct Mag: 80000x

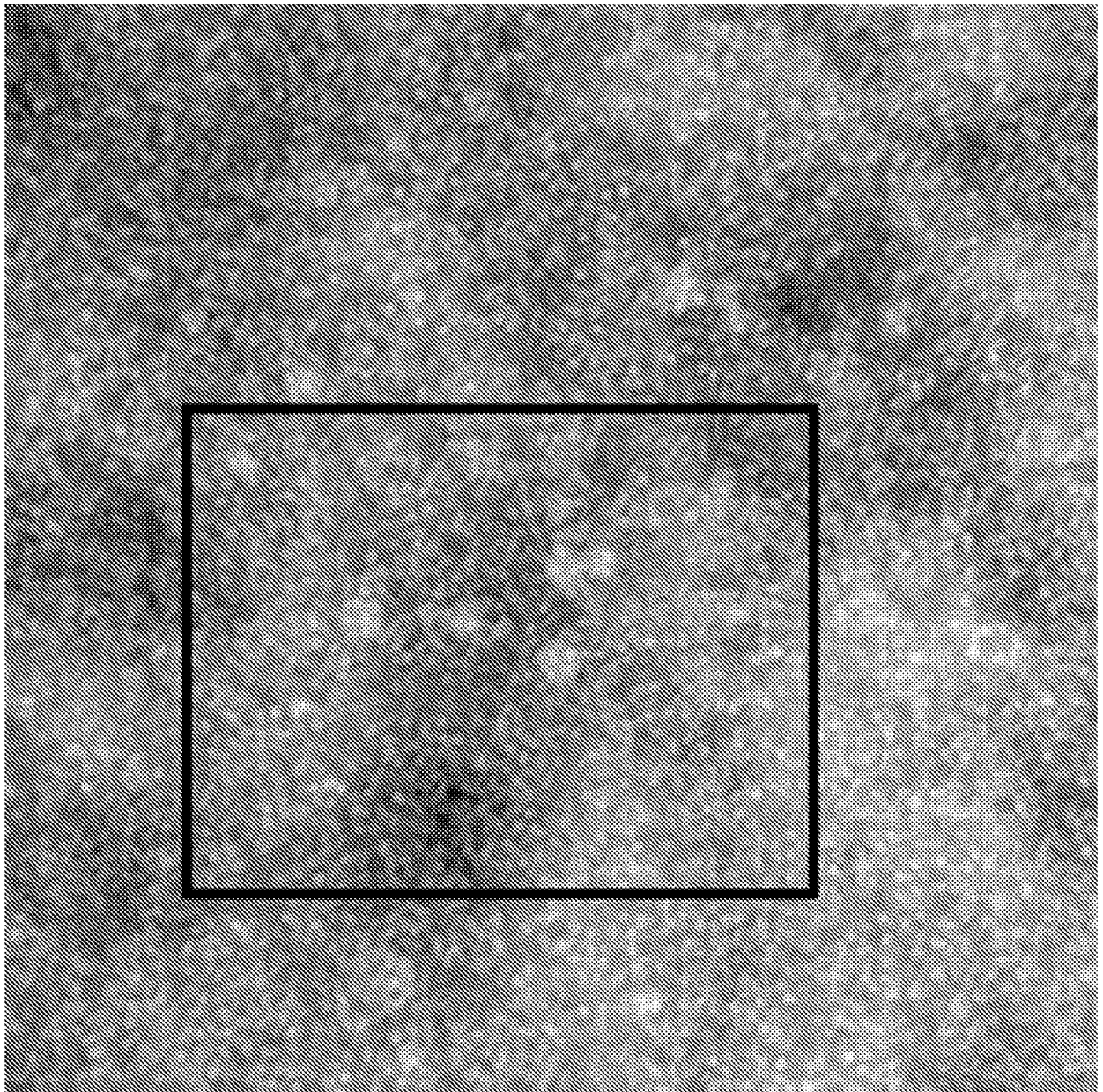
CVM EM Lab

A. Lemna-FMDV VLP from 04-DEC-2009 prep

B. Lemna-FMDV VLP from 10-FEB-2010 prep

Images taken on 11-FEB-2010

FIG. 14

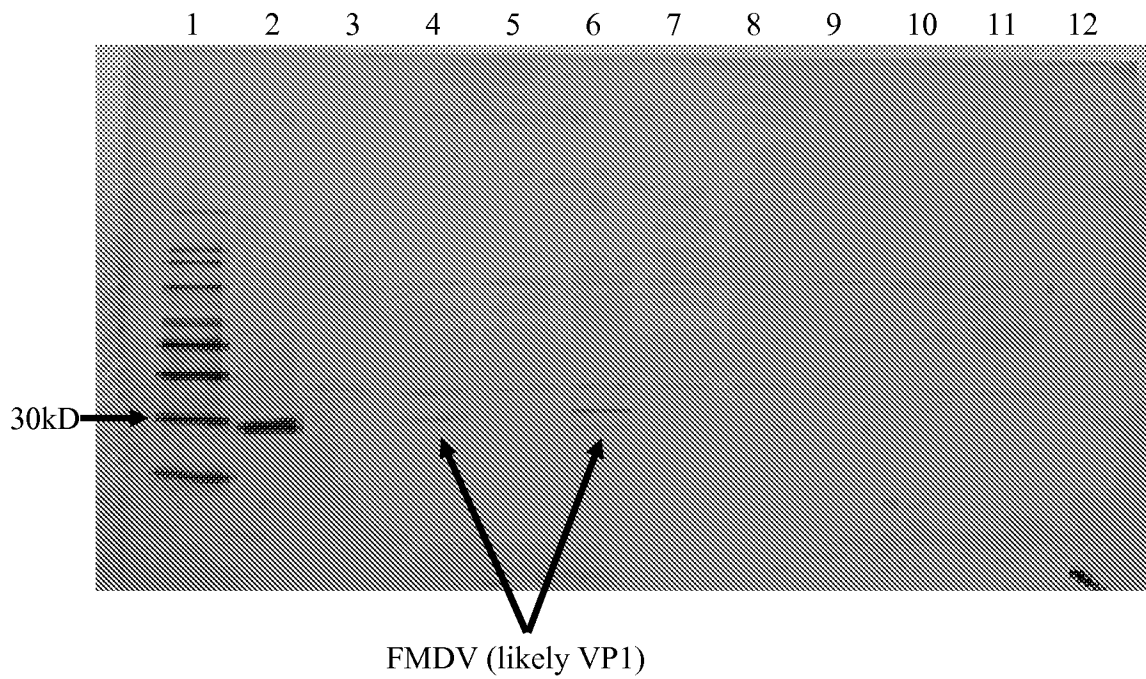


2161 Feilmeier.007  
EMDV soluble  
negative stain  
Print Mag: 195000x @ 7.0 in  
3:18:47 p 03/09/10

-----  
100 nm  
HV-100.0kV  
Direct Mag: 58000x  
CVM EM Lab

FIG. 15

WB Analysis of MerE01 and MerE03 Crude Extract



Lane	Sample
1	MW Marker
2	Inactivated FMDV
3	Lemna WT
4	E01-6
5	E01-8
6	E01-10
7	E03-8
8	E03-9
9	E03-10
10	E03-11
11	E03-12
12	Empty

FIG. 16

Schematic FMD viral particle

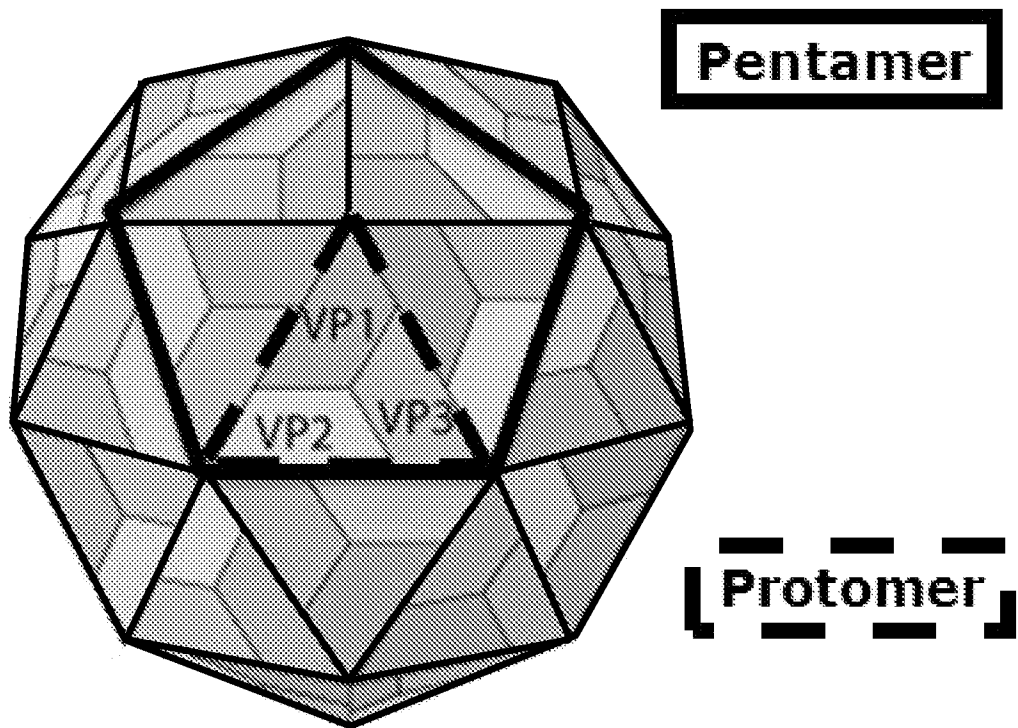
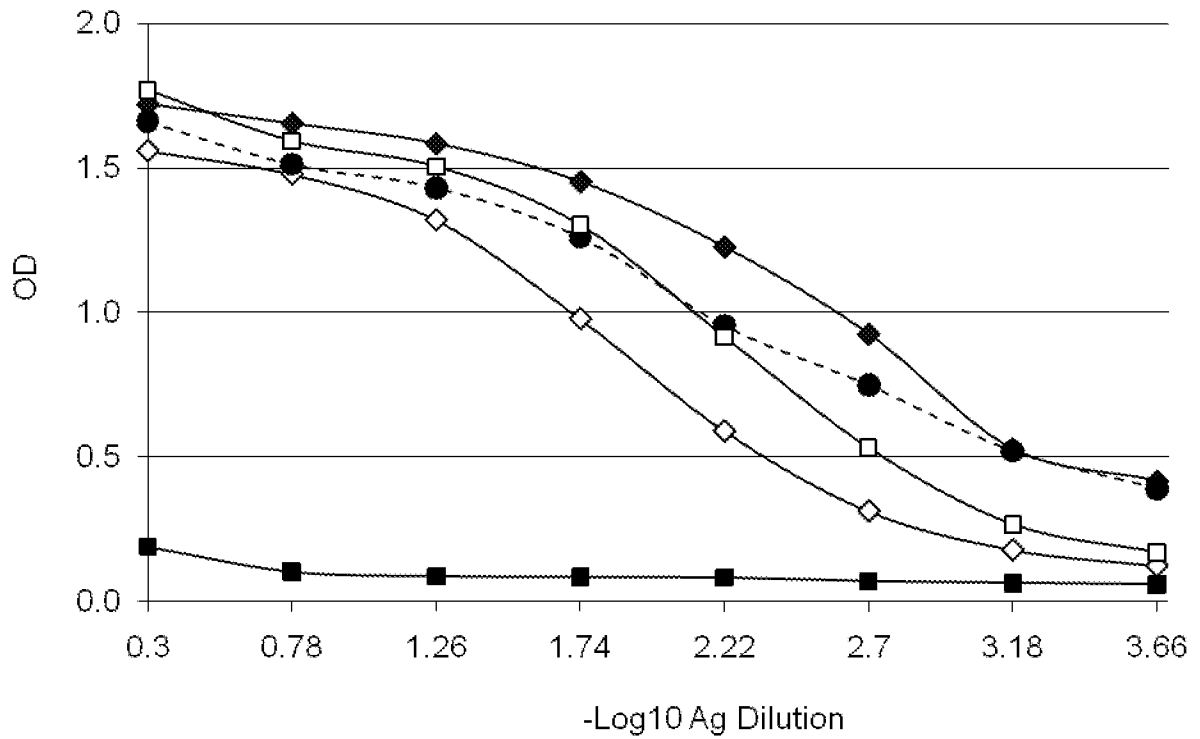


FIG. 17



◆ A24 ◇ 1FP ● 2FP □ 2 ■ Wild type

1FP: MerE01 1x crude extract  
 2FP: MerE01 5x concentrated crude extract  
 2: MerE01 10x concentrated crude extract  
 Wild type: Duckweed wild type 1x crude extract  
 AI A24: Inactivated FMDV A24



FIG. 18 (1/2)

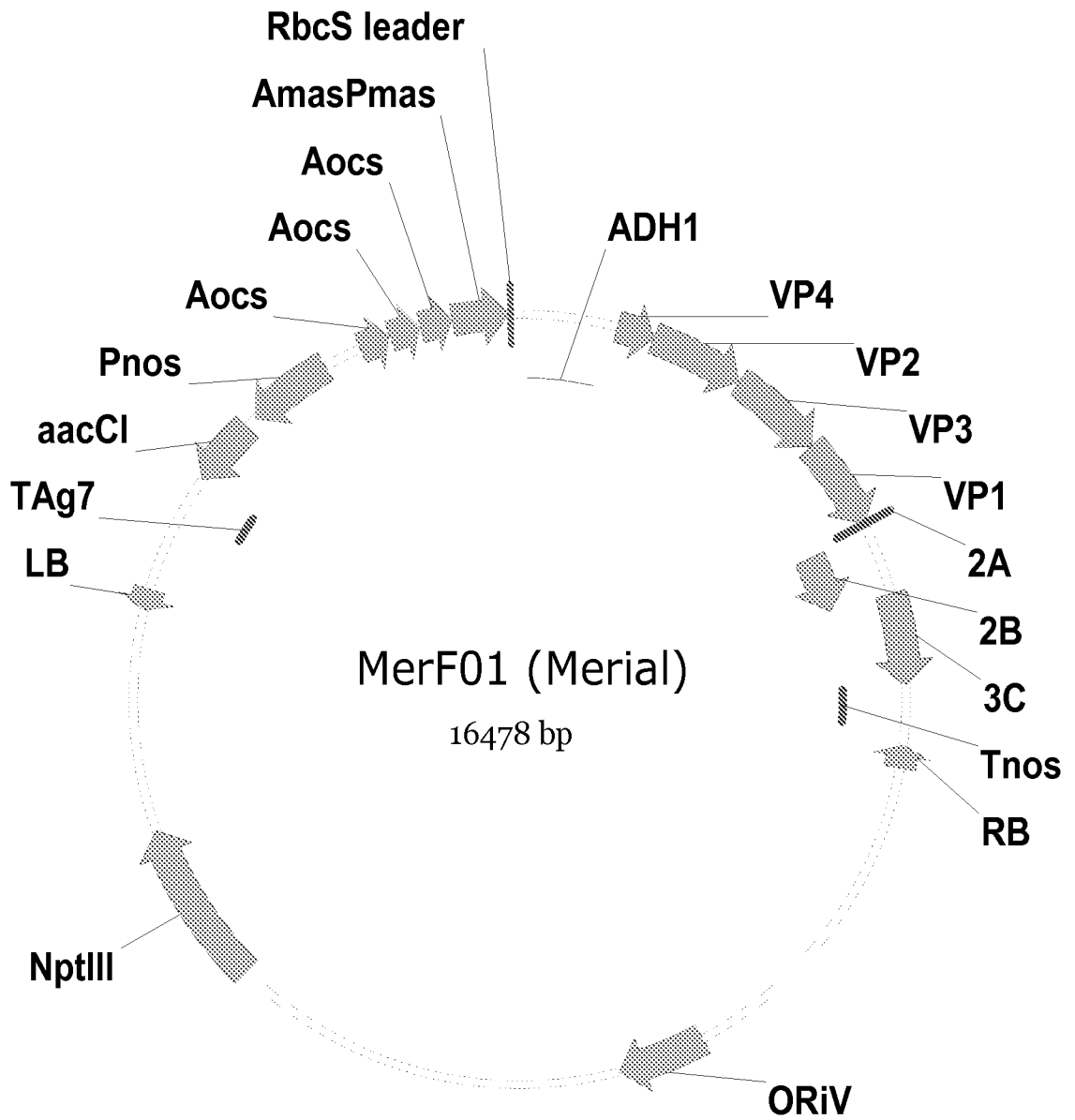


FIG. 18 (2/2)

<b>MerF01 (Merial)</b>	
<b>Feature</b>	<b>Location</b>
VP4	Start: 647 End: 904
VP2	Start: 905 End: 1558
VP3	Start: 1559 End: 2218
VP1	Start: 2219 End: 2857
2A	Start: 2858 End: 2905
2B	Start: 2906 End: 3367
3C	Start: 3368 End: 4006
NptIII	Start: 10295 End: 11443
aacCI	Start: 13950 End: 14414 (Complementary)
ADH1	Start: 37 End: 578
ORIV	Start: 6927 End: 7544
Pnos	Start: 14499 End: 15093 (Complementary)
Aocs	Start: 15358 End: 15570
Aocs	Start: 15577 End: 15789
Aocs	Start: 15796 End: 16008
AmasPmas	Start: 16015 End: 16406
Tnos	Start: 4026 End: 4281
Terminator of Gene 7 octopine synthase	Start: 13704 End: 13916 (Complementary)
Lemna gibba RbcS (SSU5B ) leader sequence	Start: 16415 End: 16477
T-DNA right border	Start: 4409 End: 4570 (Complementary)
T-DNA Left border	Start: 12969 End: 13116 (Complementary)

FIG. 19 (1/2)

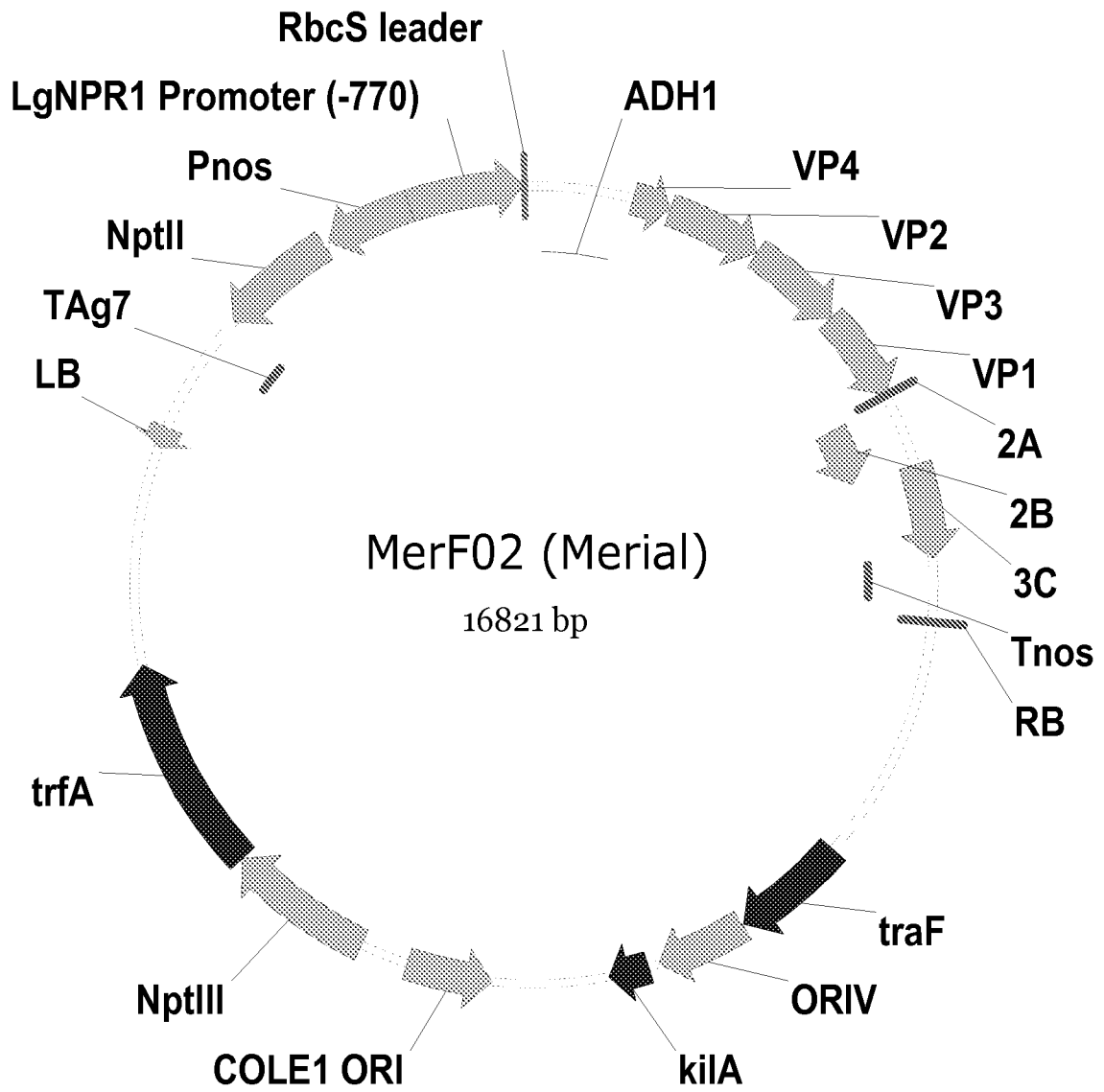


FIG. 19 (2/2)

<b>MerF02 (Merial)</b>	
<b>Feature</b>	<b>Location</b>
VP4	Start: 647 End: 904
VP2	Start: 905 End: 1558
VP3	Start: 1559 End: 2218
VP1	Start: 2219 End: 2857
2A	Start: 2858 End: 2905
2B	Start: 2906 End: 3367
3C	Start: 3368 End: 4006
NptIII	Start: 9595 End: 10587
NptII	Start: 14536 End: 15339 (Complementary)
ADH1	Start: 37 End: 578
traF	Start: 6131 End: 6914
kilA	Start: 7619 End: 7890
trfA	Start: 10588 End: 12069
Pnos	Start: 15372 End: 15967 (Complementary)
LgNPR1 Promoter (-770)	Start: 15973 End: 16749
ORIV	Start: 6927 End: 7544
COLE1_ORI	Start: 8679 End: 9264 (Complementary)
Tnos	Start: 4026 End: 4281
Terminator of Gene 7 octopine synthase	Start: 14286 End: 14511 (Complementary)
Lemna gibba RbcS (SSU5B ) leader sequence	Start: 16758 End: 16820
T-DNA right border	Start: 4435 End: 4570 (Complementary)
T-DNA Left border	Start: 13552 End: 13699 (Complementary)

FIG. 20 (1/2)

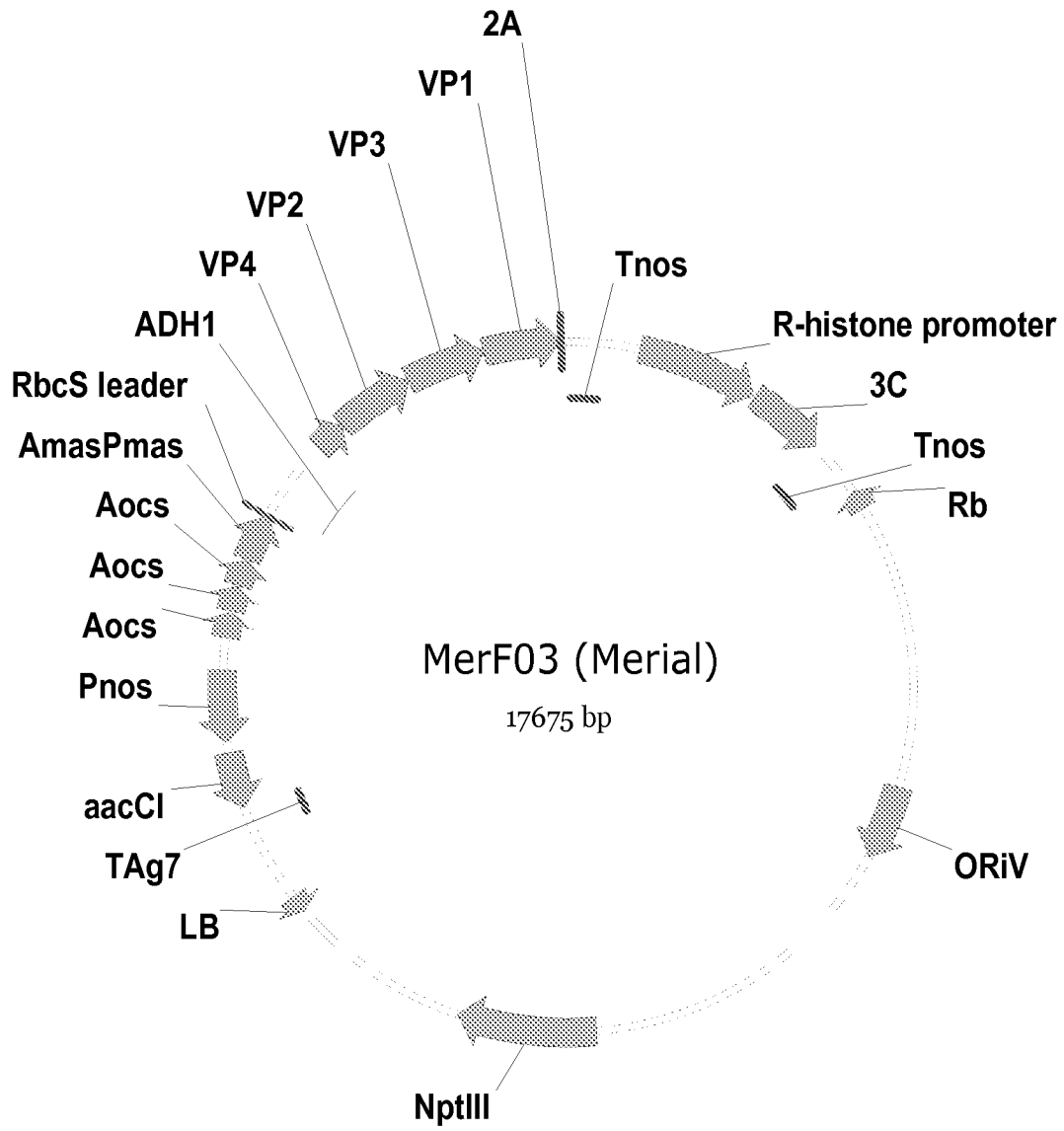


FIG. 20 (2/2)

<b>MerF03 (Merial)</b>	
<b>Feature</b>	<b>Location</b>
3C	Start: 1587 End: 2225
NptIII	Start: 8592 End: 9740
Gentamycin resistance gene	Start: 12247 End: 12711 (Complementary)
VP4	Start: 15409 End: 15666
VP2	Start: 15667 End: 16320
VP3	Start: 16321 End: 16980
VP1	Start: 16981 End: 17619
2A	Start: 17620 End: 17667
ADH1 intron	Start: 14812 End: 15353
ORiV	Start: 5224 End: 5841
L.minor replacement histone 1-kb promoter w/ putative intron.	Start: 565 End: 1568
Pnos	Start: 12796 End: 13390 (Complementary)
Aocs	Start: 13655 End: 13867
Aocs	Start: 13874 End: 14086
Aocs	Start: 14093 End: 14305
AmasPmas	Start: 14312 End: 14703
Tnos	Start: 2 End: 257
Tnos	Start: 2247 End: 2502
Terminator of Gene 7 octopine synthase	Start: 12001 End: 12213 (Complementary)
Lemna gibba RbcS (SSU5B ) leader sequence	Start: 14712 End: 14774
T-DNA right border	Start: 2706 End: 2867 (Complementary)
T-DNA Left border	Start: 11266 End: 11413 (Complementary)

FIG. 21 (1/2)

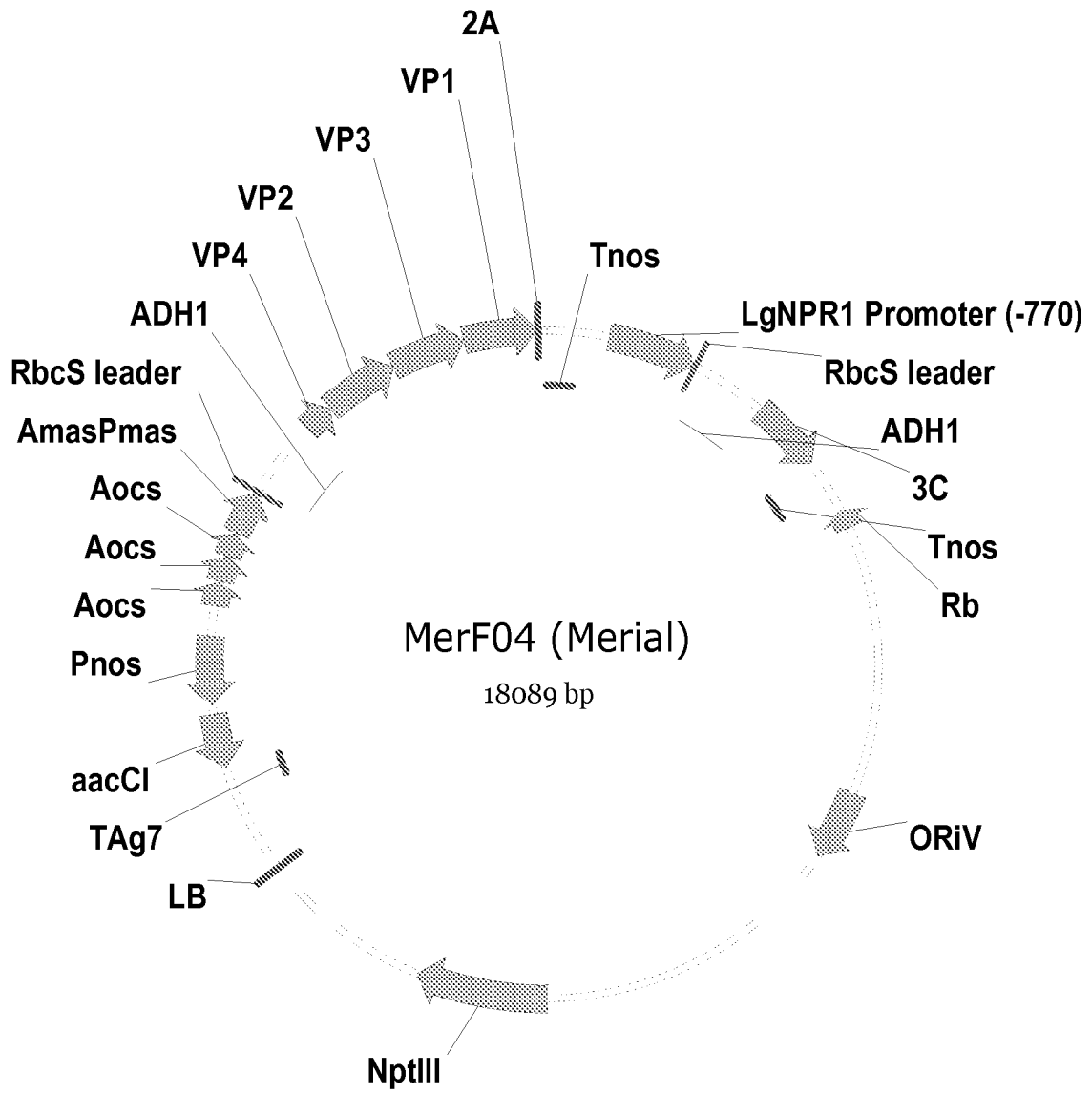


FIG. 21 (2/2)

<b>MerF04 (Merial)</b>	
<b>Feature</b>	<b>Location</b>
3C	Start: 2001 End: 2639
NptIII	Start: 9006 End: 10154
Gentamycin resistance gene	Start: 12661 End: 13125 (Complementary)
VP4	Start: 15823 End: 16080
VP2	Start: 16081 End: 16734
VP3	Start: 16735 End: 17394
VP1	Start: 17395 End: 18033
2A	Start: 18034 End: 18081
ADH1 intron	Start: 1423 End: 1964
ADH1 intron	Start: 15226 End: 15767
ORiV	Start: 5638 End: 6255
L. gibba NPR1 promoter (-770)	Start: 538 End: 1314
Pnos	Start: 13210 End: 13804 (Complementary)
Aocs	Start: 14069 End: 14281
Aocs	Start: 14288 End: 14500
Aocs	Start: 14507 End: 14719
AmasPmas	Start: 14726 End: 15117
Tnos	Start: 2 End: 257
Tnos	Start: 2661 End: 2916
Terminator of Gene 7 octopine synthase	Start: 12415 End: 12627 (Complementary)
Lemna gibba RbcS (SSU5B ) leader sequence	Start: 1323 End: 1385
Lemna gibba RbcS (SSU5B ) leader sequence	Start: 15126 End: 15188
T-DNA Right border	Start: 3120 End: 3281 (Complementary)
T-DNA Left border	Start: 11680 End: 11827 (Complementary)



FIG. 22 (1/2)

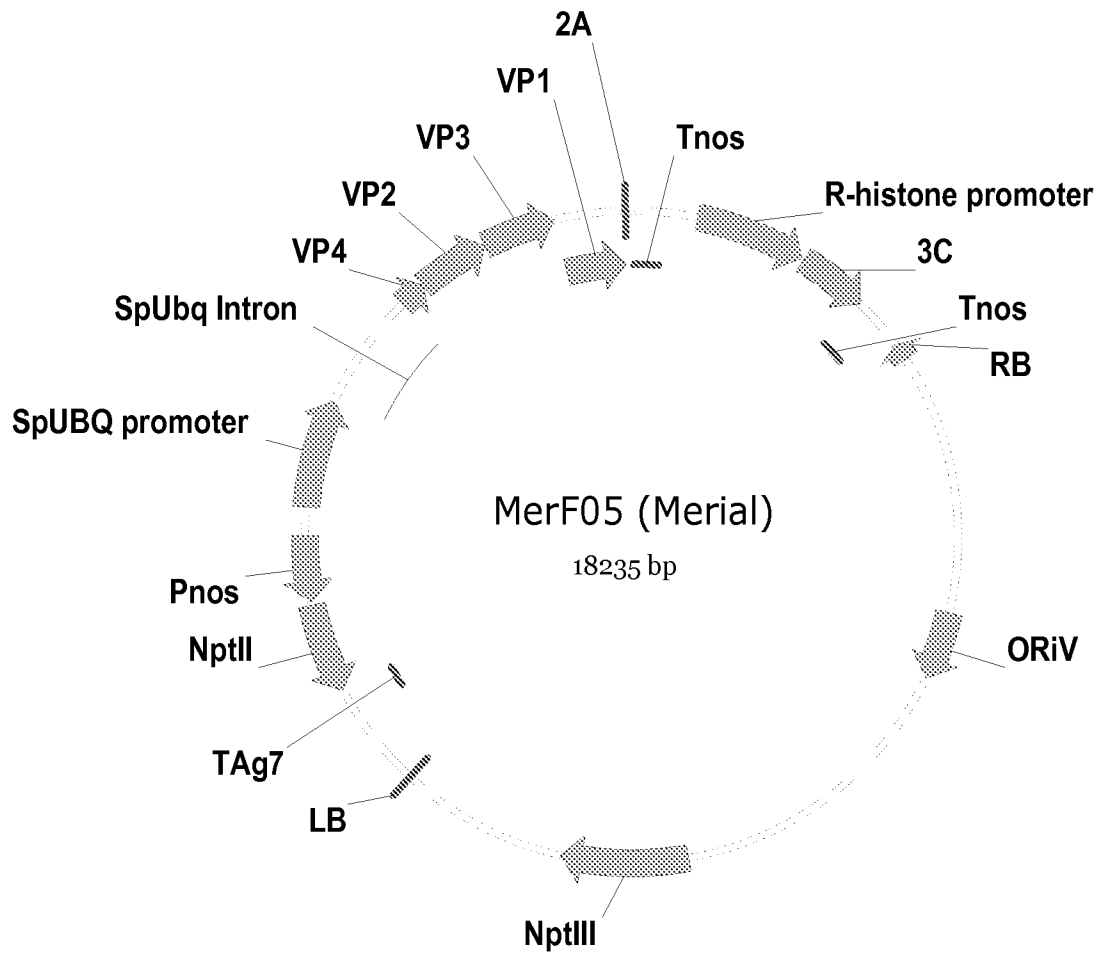


FIG. 22 (2/2)

<b>MerF05 (Merial)</b>	
<b>Feature</b>	<b>Location</b>
3C	Start: 1587 End: 2225
NptIII	Start: 8592 End: 9740
NptII	Start: 12250 End: 13053 (Complementary)
VP4	Start: 15969 End: 16226
VP2	Start: 16227 End: 16880
VP3	Start: 16881 End: 17540
VP1	Start: 17541 End: 18179
2A	Start: 18180 End: 18227
SpUbq Intron	Start: 14964 End: 15943
ORiV	Start: 5224 End: 5841
L.minor replacement histone 1-kb promoter w/ putative intron.	Start: 565 End: 1568
Pnos	Start: 13086 End: 13681 (Complementary)
SpUBQ promoter	Start: 13933 End: 14918
Tnos	Start: 2 End: 257
Tnos	Start: 2247 End: 2502
Terminator of Gene 7 octopine synthase	Start: 12000 End: 12225 (Complementary)
T-DNA Right Border	Start: 2706 End: 2867 (Complementary)
T-DNA Left border	Start: 11266 End: 11413 (Complementary)

FIG. 23 (1/2)

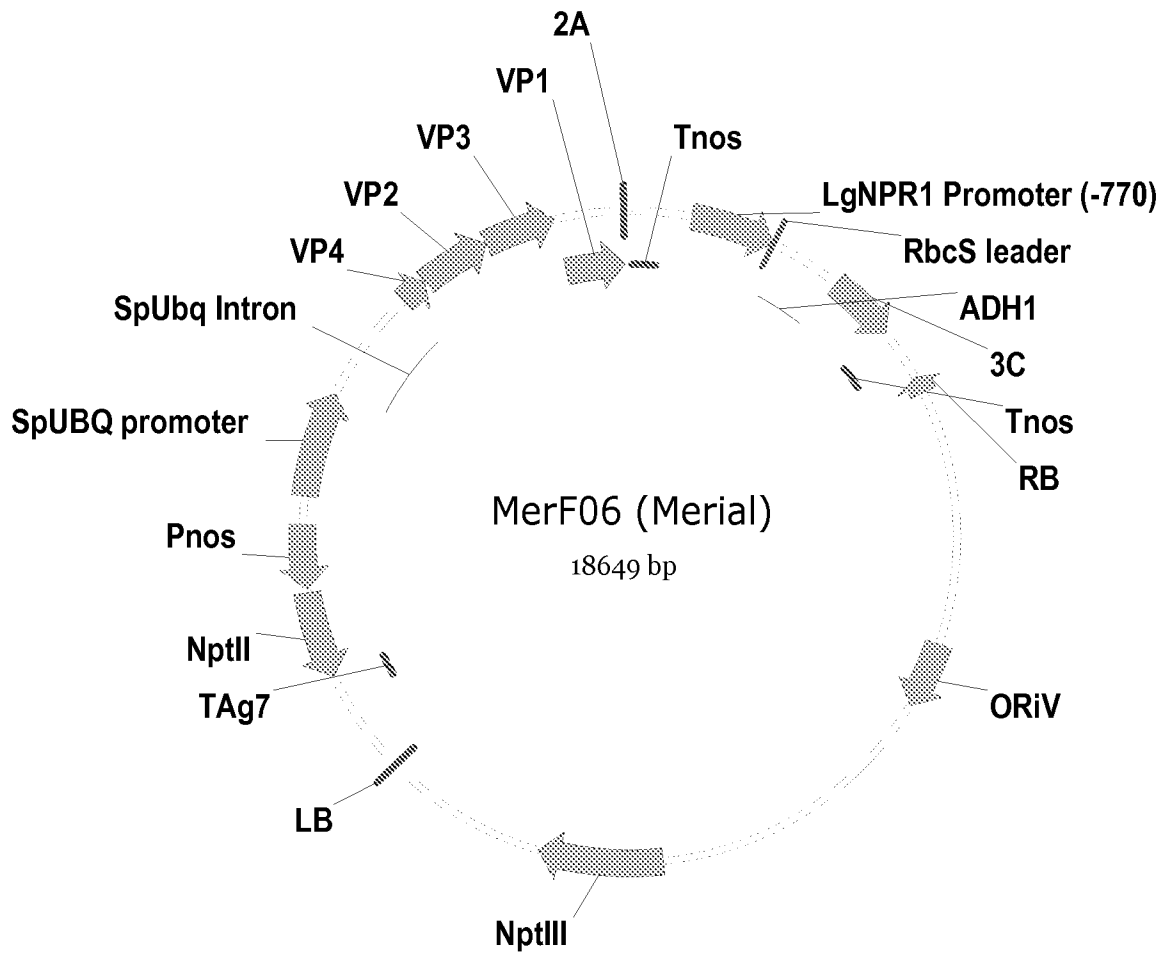


FIG. 23 (2/2)

<b>MerF06 (Merial)</b>	
<b>Feature</b>	<b>Location</b>
3C	Start: 2001 End: 2639
NptIII	Start: 9006 End: 10154
NptII	Start: 12664 End: 13467 (Complementary)
VP4	Start: 16383 End: 16640
VP2	Start: 16641 End: 17294
VP3	Start: 17295 End: 17954
VP1	Start: 17955 End: 18593
2A	Start: 18594 End: 18641
ADH1 intron	Start: 1423 End: 1964
SpUbq Intron	Start: 15378 End: 16357
ORiV	Start: 5638 End: 6255
L. gibba NPR1 promoter (-770)	Start: 538 End: 1314
Pnos	Start: 13500 End: 14095 (Complementary)
SpUBQ promoter	Start: 14347 End: 15332
Tnos	Start: 2 End: 257
Tnos	Start: 2661 End: 2916
Terminator of Gene 7 octopine synthase	Start: 12414 End: 12639 (Complementary)
Lemna gibba RbcS (SSU5B ) leader sequence	Start: 1323 End: 1385
T-DNA Right Border	Start: 3120 End: 3281 (Complementary)
T-DNA Left border	Start: 11680 End: 11827 (Complementary)