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(71) Applicant: Merial Limited [US/US]; 3239 Satellite Blvd., Duluth, GA 30096 (US).

(72) Inventor: Minke, Jules, Maarten; 16 Rue Nicolas Boileau, F-69960 Corbas (FR).

(74) Agent: Jarecki-Black, Judy; 3239 Satellite Blvd., Duluth, GA 30096 (US).

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(54) Title: REVERSE GENETICS SCHMALLENBERG VIRUS VACCINE COMPOSITIONS, AND METHODS OF USE THEREOF

(57) Abstract: The present invention relates to compositions comprising replication defective Schmallerberg virus vaccines, methods of producing the vaccines, and the administration of such vaccines to animals, including ovines and bovines. The invention further relates to methods for providing long-term protective immunity against Schmallerberg in animals, including ovines and bovines.



Reverse genetics Schmallerberg virus vaccine compositions, and methods of use thereof

INCORPORATION BY REFERENCE

This application claims priority to provisional application USSN 61/776,833, filed on March 12, 2013, which is incorporated by reference herein in its entirety. All documents cited or referenced herein, and all documents cited or referenced therein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

FIELD OF THE INVENTION

The present disclosure relates to Schmallerberg viruses (SBV), sequences and vaccine compositions. The disclosure further relates to a reverse genetics system for the production of live attenuated vaccines. It also relates to polynucleotides which can be used for the production of subunits in an *in vitro* expression system or integrated into an appropriate vehicle for *in vivo* expression. Moreover, the present disclosure relates to unmodified and modified SBV, to methods of making and using the same, and to certain DNA and protein sequences.

BACKGROUND

Schmallerberg virus (SBV) belongs to the *Orthobunyavirus* genus of the *Bunyaviridae* family. SBV was initially reported in 2001 to cause congenital malformations and stillbirths in cattle, sheep, goats, and possibly alpaca, and is apparently transmitted by midges (*Culicoides spp.*). The SBV genome comprises three segments: small (S), medium (M) and large (L). The S segment encodes the nucleocapsid (N) protein and a non-structural protein (NSs). The M genome segment encodes a non-structural protein (NSm) and two structural glycoproteins (Gn and Gc). The L segment encodes the viral polymerase (FIG. 1). Prior to the instant disclosure, the only published SBV sequence was by Hoffmann et al. (GenBank HE649912-14, herein the “FLI sequence,” Hoffmann et al., E.I.D. 18(3), 2012). It is important to note, however, that the FLI sequence was not obtained from a single virus isolate and sequence gaps were filled by sequencing of cell culture-passaged virus. Furthermore, although Hoffmann et al. claimed that the published sequences correspond to the full-length genome segments, the absence of “panhandle” sequences on the 3’ and 5’ terminal ends of the sequences made clear that the sequences were not complete. Therefore, one goal of the instant disclosure is to present a full SBV genome sequence.

SUMMARY OF THE INVENTION

The invention is based on Schmallenberg virus (SBV) vaccine compositions produced using a reverse genetics approach. Plasmids encoding the SBV S, M, and L segments were produced and co-transfected into permissive cells for production of recombinant SBV. The SBV sequence and reverse genetic system described herein may be used to produce effective, immunological and vaccine compositions. Moreover, the system naturally lends itself to the engineering of viral vaccines having little or no virulence, as compared to the wildtype SBV, thereby paving the way for safety of the host animal.

One aspect of the disclosure relates to SBV, DNA and protein sequences involved in making modified or recombinant virus. One embodiment of the invention relates to the genomic and protein sequences of SBV.

Another aspect of the disclosure relates to SBV, which have enhanced safety, strong humoral immune responses. The disclosure thus encompasses methods of making such recombinant viruses.

Another aspect of the disclosure relates to SBV vaccines or compositions having an increased level of safety compared to known SBV or other recombinant vaccines.

Another aspect of the disclosure relates to an SBV vector which provides a reverse genetics system, wherein the vector can be used as a backbone for recombinant vaccines or compositions in different host animals.

In yet another aspect, the present disclosure relates to a pharmaceutical composition or vaccine for inducing an immunological response in a host animal inoculated with the composition or vaccine, the composition or vaccine including a pharmaceutical acceptable carrier and an SB virus or viral vector. In yet another aspect of the disclosure, the SB virus or viral vector includes, within a non-essential region of the virus genome, a heterologous DNA which encodes an antigenic protein derived from a pathogen wherein the composition or vaccine when administered to a host, is capable of inducing an immunological response specific to the protein encoded by the pathogen.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF DRAWINGS

A full and enabling disclosure of the present invention, including the best mode thereof, to one of ordinary skill in the art, is set forth more particularly in the remainder of the specification, including reference to the accompanying figures, wherein:

FIG. 1 depicts structure and genome organization of Orthobunyaviruses (Source: ViralZone 2010, Swiss Institute of Bioinformatics). The sizes of the SBV L, M and S segments are indicated;

FIG. 2 is a bright-field micrograph of mock-infected Vero cells (A) and cells inoculated with SBV rescued on BSR-T7/5 cells (B). Mock-infected (C) and SBV-infected (D) Vero cells stained with an anti-SBV convalescent sheep serum;

FIG. 3 presents the SBV S segment and RevGen construct features;

FIG. 4 presents the SBV M segment and RevGen construct features;

FIG. 5 presents the SBV L segment and RevGen construct features;

FIG. 6 presents the published HE649912 sequence.

DETAILED DESCRIPTION OF THE INVENTION

Other objects, features and aspects of the present invention are disclosed in, or are obvious from, the following Detailed Description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only and is not intended as limiting the broader aspects of the present invention, which broader aspects are embodied in the exemplary construction. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment can be used in another embodiment to yield a still further embodiment. It is intended that the present invention cover such modifications and variations as come within the scope of the appended claims and their equivalents. The contents of all references, published patents, and patents cited throughout the present application are hereby incorporated by reference in their entirety.

In a first aspect, the present disclosure provides vaccine compositions comprising SBV produced using a reverse genetic approach. The composition comprises nucleotides encoding S, M, and L viral segments. In an embodiment, the S, M, and L segments encode sequences having at least 90% homology to the sequences or reverse complementary sequences of those set forth

in SEQ ID NO:3, 9, or 14.

In an embodiment, the disclosure provides an immunological composition comprising a Schmallenberg virus (SBV) or portion thereof. The composition may comprise SBV S, M, and L segments.

In an embodiment, the SBV comprises polynucleotides having at least 90% sequence identity to polynucleotides having the sequence as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).

In an embodiment, the compositions comprise SBV comprising a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to the polynucleotide having the sequences as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment). In another embodiment, the S segment encodes a peptide having at least 90% homology to SEQ ID NO:4; the M segment encodes a peptide having at least 90% homology to SEQ ID NO:10 or 11; and the L segment encodes a peptide having at least 90% homology to SEQ ID NO:15. In yet another embodiment, the S, M, and L segments encode peptides as set forth in SEQ ID NO:4, SEQ ID NOs:10 or 11, and SEQ ID NO:15, respectively.

In an embodiment, the composition is a vaccine composition, and further comprises a veterinarily and/or pharmaceutically acceptable carrier.

The disclosure also provides a reverse genetics (RG) system for producing the immunological or vaccine compositions comprising SV virus or viral sequences. The system may comprise one or more plasmids comprising nucleotides having at least 90% homology to the sequence of SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).

In an embodiment, the system of comprises nucleotides having the sequences as set forth in SEQ ID NOs:3, 9, and 14. The RG system may comprise 3 separate plasmids, wherein a first plasmid encodes the S segment, a second plasmid encodes the M segment, and a third plasmid encodes the L segment.

In another embodiment of the RG system, the S, M, and L segments may encode peptides as set forth in SEQ ID NOs:4 (S segment), 10 or 11 (M segment), and 15 (L segment).

In an embodiment of the RG system, the first plasmid encodes the SBV S segment 5' and 3' UTR, the second plasmid encodes the SBV M segment 5' and 3' UTR, and the third plasmid encodes the SBV L segment 5' and 3' UTR. In another embodiment, the first plasmid may

comprise the sequences as set forth in SEQ ID NOs:2 & 3; the second plasmid may comprise the sequences as set forth in SEQ ID NOs: 8 & 12 or 13; and the third plasmid may comprise the sequences as set forth in SEQ ID NOs:13 & 16.

In another embodiment, the RG system plasmids comprise a T7 minimal promoter as set forth in SEQ ID NO:7 and a ribozyme / T7 terminator as set forth in SEQ ID NO:6.

The disclosure also provides for cDNA(s) useful for the production of the SBV compositions. The cDNA(s) may comprise nucleotides coding for SBV S, M, and L segments. The cDNA(s) may comprise nucleotides having at least 90% homology to the sequences as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).

In an aspect, the disclosure provides isolated RNA molecules transcribed from the cDNA(s) used for producing the SB viruses and compositions. In an embodiment, the RNA molecules comprise sequence complementary to SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment), or to sequences having at least 90% homology to the complementary sequences of those set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).

In another aspect, the disclosure provides a method for producing the SB viruses and compositions, comprising the steps of:

- a. producing a cDNA encoding each of the SBV segments (S, M, and L);
- b. co-transfecting the cDNAs into cells capable of producing the SBV from said cDNAs; and
- c. allowing the cells to make the virus, thereby producing the virus.

In an embodiment of the method, the cDNAs have the sequence as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment), or the sequence having at least 90% homology thereto.

In another embodiment of the method, the cells are BSR-T7/5 cells.

In still another aspect, the disclosure provides a method for providing an animal protection against SBV comprising the steps of administering the SB viruses and/or compositions to said animal, thereby providing the protection. The viruses or compositions may be administered as an IP or SC dose, and in a range of about 10µg to about 300µg per dose. In an embodiment, the protection lasts for at least about 1 year.

For convenience, certain terms employed in the Specification, Examples, and appended Claims are collected here.

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.

It is also noted that in this disclosure and particularly in the claims, terms such as “comprises”, “comprised”, “comprising” and the like can have the meaning attributed to such terms 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 by U.S. Patent law, *e.g.*, they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

As used herein, the term “animal” includes all vertebrate animals including humans. 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 includes an individual animal in all stages of development, including embryonic and fetal stages.

As used herein, the term “virulent” means an isolate that retains its ability to be infectious in an animal host.

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

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

As used herein, the term “immune response” refers to a response elicited in an animal. An immune response may refer to cellular immunity (CMI); humoral immunity or may involve both. The present invention also contemplates a response limited to a part of the immune system. For example, a vaccine composition of the present invention may specifically induce an increased gamma interferon response.

As used herein, the term “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 protein, a polypeptide, a peptide, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

As used herein, the term “multivalent” means a vaccine containing more than one antigen whether from the same species (*i.e.*, different isolates of SBV *serotypes*), from a different species (*i.e.*, isolates from both *bovine diarrhea virus* and *bovine BTV*), or a vaccine containing a combination of antigens from different genera (for example, a vaccine comprising antigens from *leptospira spp.*, *BTV*, *lyme disease*, and *parainfluenza*).

As used herein, the term “adjuvant” means a substance added to a vaccine to increase a vaccine’s immunogenicity, as compared with its efficacy in absence of the adjuvant. The mechanism of how adjuvants operate is not entirely known. Some adjuvants are believed to enhance the immune response by slowly releasing the antigen, while other adjuvants are strongly immunogenic in their own right and are believed to function synergistically. Known vaccine adjuvants include, but are not limited to, oil and water emulsions (for example, complete Freund's adjuvant and incomplete Freund's adjuvant, and adjuvants disclosed in US patent numbers US7371395 to Merial Limited, which are herein incorporated by reference in their entirety), *Corynebacterium parvum*, Bacillus Calmette Guerin, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, certain synthetic polymers such as poly amino acids and co-polymers of amino acids, saponin, "REGRESSIN" (Vetrepharm,

Athens, Ga.), "AVRIDINE" (N, N-dioctadecyl-N',N'-bis(2-hydroxyethyl)-propanediamine), paraffin oil, muramyl dipeptide and the like.

As used herein, the term "emulsion" refers to a combination of at least two substances, wherein a first substance is dispersed in a second substance in which the first substance is insoluble. One example of an emulsion of the present invention is an oil phase dispersed in an aqueous phase.

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

As used herein, the term "vaccine composition" includes at least one antigen or immunogen in a pharmaceutically acceptable vehicle useful for inducing an immune response in a host. Vaccine compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species and condition of the recipient animal, and the route of administration. The route of administration can be percutaneous, via mucosal administration (*e.g.*, oral, nasal, anal, vaginal) or via a parenteral route (intradermal, intramuscular, subcutaneous, intravenous, or intraperitoneal). Vaccine compositions can be administered alone, or can be co-administered or sequentially administered with other treatments or therapies. Forms of administration may include suspensions, syrups or elixirs, and preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (*e.g.*, injectable administration) such as sterile suspensions or emulsions. Vaccine compositions may be administered as a spray or mixed in food and/or water or delivered in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard pharmaceutical texts, such

as “Remington's Pharmaceutical Sciences,” 1990 may be consulted to prepare suitable preparations, without undue experimentation.

The term “purified” as used herein does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified immunogen preparation, such as protein or inactivated virus, is one in which the immunogen is more enriched than the immunogen is in its natural environment. An immunogen preparation is herein broadly referred to as “purified” such that the immunogen represents at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, of the total immunogen content of the preparation. A “crude preparation”, which represents the lowest degree of purification, may contain as little as less than 60%, less than 20%, less than 10%, less than 5%, or less than 1% of immunogenic components.

The term “highly purified” as used herein is intended to suggest a “higher degree of purity” as compared to the term “moderately purified”. This “higher degree of purity” can include, but is in no way limited to, reduced percentages of contaminants, in an immunological preparation that has been “highly purified” versus an immunological preparation that has been “moderately purified”. As discussed herein, “highly purified” immunological preparations will have the lowest to undetectable percentages of contaminants that can cause: reduced desired immune response, increased undesired immune response (e.g. hypersensitivity reaction), or reduced formulation stability. Similarly, an immunological preparation that has been “moderately purified” contains relatively reduced percentages of contaminants versus an immunological preparation that has been “minimally purified”, which likewise, has reduced percentages of contaminants versus a preparation designated a “crude preparation”.

Contaminants in an immunological preparation can include, but are in no way limited to, substances that contribute negatively to an immunological composition according to the present invention. One of several examples of a contaminant contributing negatively would be a contaminant that reduces the ability of an immunological composition of the present invention to elicit an immune response in animals.

Varying levels of purity (e.g. “highly purified”, “moderately purified”, and the like) can be achieved using various methods. For example, a combination of chromatography and size exclusion gel filtration can result in a “highly purified” or “moderately purified” immunological preparations. Differences in source/type of immunogens, as well as slight variations in purification procedures can significantly affect the final degree of immunogen purity. In general,

as used herein, immunological preparations having the lowest to highest percentage of contaminants will be described as 1) “highly purified”, 2) “moderately purified”, 3) “minimally purified”, 4) “crude preparation”, respectively. A “highly purified” preparation will have the lowest level across all types of contaminants. A “moderately purified” preparation will have relatively low levels of most types of contaminants, but may have one type of contaminant in higher abundance than would be observed for a comparable “highly purified” preparation. Likewise, a “minimally purified preparation” will have relatively low levels of some types of contaminants, but may have more than one type of contaminant in higher abundance than a comparable “moderately purified” preparation. As expected, a “crude preparation” has the highest level of contaminants, across all contaminant types, as compared to the other types of preparations discussed herein.

A “polynucleotide” is a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and analogs in any combination. Polynucleotides may have three-dimensional structure, and may perform any function, known or unknown. The term “polynucleotide” includes double-, single-stranded, and triple-helical molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double stranded form and each of two complementary forms known or predicted to make up the double stranded form of either the DNA, RNA or hybrid molecule.

The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant 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 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.

An “isolated” polynucleotide or polypeptide is one that is substantially free of the materials with which it is associated in its native environment. By substantially free, is meant at least 50%, advantageously at least 70%, more advantageously at least 80%, and even more advantageously at least 90% free of these materials.

The invention further comprises a complementary strand to a SBV polynucleotide.

The complementary strand can be polymeric and of any length, and can contain deoxyribonucleotides, ribonucleotides, and analogs in any combination.

Hybridization reactions can be performed under conditions of different “stringency.” Conditions that increase stringency of a hybridization reaction are well known. See for examples, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook *et al.* 1989). Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25 °C., 37 °C., 50 °C., and 68 °C.; buffer concentrations of 10×SSC, 6×SSC, 1×SSC, 0.1×SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalent using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2 or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6×SSC, 1×SSC, 0.1×SSC, or deionized water.

The invention further encompasses polynucleotides encoding functionally equivalent variants and derivatives of a SBV polypeptides and functionally equivalent fragments thereof which may enhance, decrease or not significantly affect properties of the polypeptides encoded thereby. These functionally equivalent variants, derivatives, and fragments display the ability to retain SBV activity. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. Conservative amino acid substitutions are glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid / glutamic acid; serine/threonine/methionine; lysine/arginine; and phenylalanine / tyrosine / tryptophan.

For the purposes of the present invention, sequence identity or homology is determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical algorithms. A non-limiting example of a mathematical algorithm used

for comparison of two sequences is the algorithm of Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1990;87: 2264-2268, modified as in Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1993;90: 5873-5877.

Another example of a mathematical algorithm used for comparison of sequences is the algorithm of Myers & Miller, CABIOS 1988; 4: 11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Yet another useful algorithm for identifying regions of local sequence similarity and alignment is the FASTA algorithm as described in Pearson & Lipman, Proc. Natl. Acad. Sci. USA 1988;85: 2444-2448.

Advantageous for use according to the present invention is the WU-BLAST (Washington University BLAST) version 2.0 software. WU-BLAST version 2.0 executable programs for several UNIX platforms can be downloaded from <ftp://blast.wustl.edu/blast/executables>. This program is based on WU-BLAST version 1.4, which in turn is based on the public domain NCBI-BLAST version 1.4 (Altschul & Gish, 1996, Local alignment statistics, Doolittle ed., Methods in Enzymology 266: 460-480; Altschul *et al.*, Journal of Molecular Biology 1990;215: 403-410; Gish & States, 1993; Nature Genetics 3: 266-272; Karlin & Altschul, 1993; Proc. Natl. Acad. Sci. USA 90: 5873-5877; all of which are incorporated by reference herein).

In general, comparison of amino acid sequences is accomplished by aligning an amino acid sequence of a polypeptide of a known structure with the amino acid sequence of a the polypeptide of unknown structure. Amino acids in the sequences are then compared and groups of amino acids that are homologous are grouped together. This method detects conserved regions of the polypeptides and accounts for amino acid insertions and deletions. Homology between amino acid sequences can be determined by using commercially available algorithms (see also the description of homology above). In addition to those otherwise mentioned herein, mention is made too of the programs BLAST, gapped BLAST, BLASTN, BLASTP, and PSI-BLAST, provided by the National Center for Biotechnology Information. These programs are widely used in the art for this purpose and can align homologous regions of two amino acid sequences.

In all search programs in the suite the gapped alignment routines are integral to the database search itself. Gapping can be turned off if desired. The default penalty (Q) for a gap of length one is Q=9 for proteins and BLASTP, and Q=10 for BLASTN, but may be changed to any

integer. The default per-residue penalty for extending a gap (R) is R=2 for proteins and BLASTP, and R=10 for BLASTN, but may be changed to any integer. Any combination of values for Q and R can be used in order to align sequences so as to maximize overlap and identity while minimizing sequence gaps. The default amino acid comparison matrix is BLOSUM62, but other amino acid comparison matrices such as PAM can be utilized.

Alternatively or additionally, the term “homology” or “identity”, for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence homology can be calculated as $(N_{\text{ref}} - N_{\text{dif}}) * 100 / N_{\text{ref}}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC ($N_{\text{ref}}=8$; $N_{\text{dif}}=2$).

Alternatively or additionally, “homology” or “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 & Lipman, Proc Natl Acad Sci USA 1983;80:726, incorporated herein by reference), 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.

And, without undue experimentation, the skilled artisan can consult with many other programs or references for determining percent homology.

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 heterologous polynucleotide may comprise a sequence of interest for purposes of therapy, and may optionally be in the form of an expression cassette. As used herein, a vector need not be

capable of replication in the ultimate target cell or subject. The term includes cloning vectors for translation of a polynucleotide encoding sequence. Also included are viral vectors.

The term “recombinant” means a polynucleotide of genomic cDNA, semisynthetic, or synthetic origin 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.

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, 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 embodiments of this invention.

Methods for making and/or administering a vector or recombinants or plasmid for expression of gene products of genes of the invention either in vivo or in vitro can be any desired method, e.g., a method which is by or analogous to the methods disclosed in, or disclosed in documents cited in: U.S. Pat. Nos. 4,603,112; 4,769,330; 4,394,448; 4,722,848; 4,745,051; 4,769,331; 4,945,050; 5,494,807; 5,514,375; 5,744,140; 5,744,141; 5,756,103; 5,762,938; 5,766,599; 5,990,091; 5,174,993; 5,505,941; 5,338,683; 5,494,807; 5,591,639; 5,589,466; 5,677,178; 5,591,439; 5,552,143; 5,580,859; 6,130,066; 6,004,777; 6,130,066; 6,497,883; 6,464,984; 6,451,770; 6,391,314; 6,387,376; 6,376,473; 6,368,603; 6,348,196; 6,306,400; 6,228,846; 6,221,362; 6,217,883; 6,207,166; 6,207,165; 6,159,477; 6,153,199; 6,090,393; 6,074,649; 6,045,803; 6,033,670; 6,485,729; 6,103,526; 6,224,882; 6,312,682; 6,348,450 and 6,312,683; U.S. patent application Ser. No. 920,197, filed Oct. 16, 1986; WO 90/01543; WO91/11525; WO 94/16716; WO 96/39491; WO 98/33510; EP 265785; EP 0 370 573; Andreansky *et al.*, Proc. Natl. Acad. Sci. USA 1996;93:11313-11318; Ballay *et al.*, EMBO J. 1993;4:3861-65; Felgner *et al.*, J. Biol. Chem. 1994;269:2550-2561; Frolov *et al.*, Proc. Natl.

Acad. Sci. USA 1996;93:11371-11377; Graham, Tibtech 1990;8:85-87; Grunhaus *et al.*, Sem. Virol. 1992;3:237-52; Ju *et al.*, Diabetologia 1998;41:736-739; Kitson *et al.*, J. Virol. 1991;65:3068-3075; McClements *et al.*, Proc. Natl. Acad. Sci. USA 1996;93:11414-11420; Moss, Proc. Natl. Acad. Sci. USA 1996;93:11341-11348; Paoletti, Proc. Natl. Acad. Sci. USA 1996;93:11349-11353; Pennock *et al.*, Mol. Cell. Biol. 1984;4:399-406; Richardson (Ed), Methods in Molecular Biology 1995;39, "Baculovirus Expression Protocols," Humana Press Inc.; Smith *et al.* (1983) Mol. Cell. Biol. 1983;3:2156-2165; Robertson *et al.*, Proc. Natl. Acad. Sci. USA 1996;93:11334-11340; Robinson *et al.*, Sem. Immunol. 1997;9:271; and Roizman, Proc. Natl. Acad. Sci. USA 1996;93:11307-11312. Thus, the vector in the invention can be any suitable recombinant virus or virus vector, such as a poxvirus (e.g., vaccinia virus, avipox virus, canarypox virus, fowlpox virus, raccoonpox virus, swinepox virus, etc.), adenovirus (e.g., canine adenovirus), herpesvirus, baculovirus, retrovirus, etc. (as in documents incorporated herein by reference); or the vector can be a plasmid.

It is understood to one of skill in the art that conditions for culturing a host cell varies according to the particular gene and that routine experimentation is necessary at times to determine the optimal conditions for culturing SBV depending on the host cell. 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.

Polynucleotides comprising a desired sequence can be inserted into a suitable cloning or expression vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides can be introduced into host cells by any means known in the art. The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including direct uptake, endocytosis, transfection, f-mating, electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is infectious, for instance, a retroviral vector). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

Advantageously, the pharmaceutical and/or therapeutic compositions and/or formulations according to the invention comprise or consist essentially of or consist of an effective quantity to

elicit a therapeutic response of one or more expression vectors and/or 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.

One skilled in the art can determine the effective plasmid dose to be used for each immunization or vaccination protocol and species from this disclosure and the knowledge in the art.

Also in connection with such a therapeutic composition, from the disclosure 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.

In some embodiments, non-ionic hydrophilic surfactants having a high hydrophilic-lipophilic balance (HLB) value may be added to the disclosed formulations. This group comprises ethoxylated fatty acid monoesters of sorbitan (in particular 20 ethoxyl groups) (e.g. ethoxylated sorbitan monolaurate such as TWEEN 20[®], ethoxylated sorbitan monopalmitate such as TWEEN 40[®], ethoxylated sorbitan monostearate (such as TWEEN 60[®], ethoxylated sorbitan monooleate such as TWEEN 80[®], ethoxylated fatty alcohols (in particular 15-30 ethoxyl groups) (e.g. BRIJ 78[®], BRIJ 98[®], BRIJ 721[®]), ethoxylated fatty acids (in particular 15-30 ethoxyl groups) (e.g. MYRJ 49[®], MYRJ 51[®], MYRJ 52[®], MYRJ 53[®]), non-ionic block-copolymers (e.g. polyoxyethylene/ polyoxypropylene copolymer (POE-POP), such as LUTROL F127[®], LUTROL F68[®]), and combinations thereof.

The disclosed formulation may include fatty acid esters of sorbitan (e.g. sorbitan monolaurate, like SPAN 20[®], sorbitan monopalmitate, such as SPAN 40[®], sorbitan monostearate, such as SPAN 60[®], sorbitan tristearate, such as SPAN 65[®], sorbitan monooleate, like SPAN 80[®], sorbitan trioleate, like SPAN 85[®], sorbitan monoisostearate, such as ARLACEL 987[®], sorbitan isostearate, such as CRILL 6[®]), fatty acid esters of mannide (e.g. MONTANIDE 80[®], mannide monooleate (such as ARLACEL A[®]), mannide dioleate, mannide trioleate, mannide tetraoleate), ethoxylated fatty acid esters of mannide (2, 3 or 4 ethoxyl groups) (e.g. MONTANIDE 888[®], MONTANIDE 103[®], ethoxylated mannide monooleate, ethoxylated mannide dioleate, ethoxylated mannide trioleate, ethoxylated mannide tetraoleate), and combinations thereof. The fatty acid may be oleate, palmitate, stearate, isostearate, laurate and combinations thereof.

In some embodiments, oils may be added to the disclosed formulations, including mineral oils, such as paraffin oil including isoparaffinic oil and/or naphthenic oil, squalane, pristane, polyisobutene oil, hydrogenated polyisobutene oil, polydecene oil, polyisoprene oil, polyisopropene oil and the like. Such oils may, for example, be those marketed under the name "MARCOL 52[®]" or "MARCOL 82[®]" (produced by Esso, France) or "DRAKEOL 6VR[®]" or "DRAKEOL 5[®]" "DRAKEOL 7[®]" (produced by Penreco, USA), "CLEAROL[®]" (produced by Sonneborn, USA), "Paraffin Oil Codex AAB2[®]" (produced by Aiglon, France), BLANDOL (produced by Sonneborn, USA), ONDINA 915 (produced by Shell, UK). The oil may also be a mixture of oils comprising at least 2 oils selected among the oils described herein, and in any proportion. The mixture of oils may also comprise at least one oil selected among the oils described above and at least one vegetable oil, and this vegetable oil represents from about 0.1% to about 33% of the oily phase, preferably from about 10% to about 25% v/v. These vegetable oils are unsaturated oils rich in oleic acid that are biodegradable and preferably liquid at the storage temperature (about +4 °C.) or at least make it possible to give emulsions that are liquid at this temperature. For example the vegetable oil may be groundnut oil, nut oil, sunflower oil, safflower oil, soya oil, onager oil and the like.

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

EXAMPLES

Example 1 – Complete SBV genome sequence and comparison to published sequence.

SBV was isolated from the brain of a malformed lamb and initially grown on BHK cells. Although cytopathic effect (cpe) was observed on BHK cells, the virus was lost upon passage of supernatant, so further virus production was carried out using Vero cells. The full genome sequence of the lamb isolate was determined by "next generation sequencing" (NGS) of passage #2 (Vero cells) using a single Miseq run. This analysis suggested (later confirmed, see below) that HE649912.1 (GenBank accession number; SEQ ID NO:17), representing the L segment, lacks 12 nucleotides on the 3' untranslated region (UTR) and 6 nucleotides on the 5' UTR; HE649913.1, representing the M genome segment, lacks 12 nucleotides on the 3' UTR and a sequence duplication resulting from a sequencing artifact on the 5' UTR; HE649914.1, representing the S genome segment, lacks 54 nucleotides on the 5' UTR and 9 nucleotides on the 3' UTR.

In addition, four amino acid differences between cow blood (CB, FLI sequence) and lamb brain sequences (LB, sequence of instant disclosure) were identified in the L protein: H272Y; D487N; Y1019H; E1159D (Y, N, H, D corresponds to the LB sequence). A total of 12 amino acid differences were identified in the sequence of the glycoprotein precursor (GPC): C352R; T538A; N581I; H587D; N629Y; E690K; T737I; H738R; P739L; K746E; N1159D; K1340E. Some silent nucleotide differences were also identified (a potentially relevant silent mutation is detailed below).

Example 2 – Infection of sheep with SBV.

Initial experiments were conducted to determine the virulence of the LB isolate in sheep. Since neither clinical signs nor viremia were detected, sheep were then inoculated with SBV isolated from bovine. Although no clinical signs were observed, viremia was detected by quantitative PCR. Subsequent experiments with sheep confirmed this finding and so the LB isolate was not pursued (recent sequencing results suggest that the LB isolate is an “atypical” SBV and that the sequence differences are not correlated with the host species).

Example 3 – Sequence of SBV isolated from Cattle Blood

The full genome sequence of SBV isolated at the CVI from cattle blood was determined by Illumina sequencing and was found to be highly similar to the (corrected) FLI sequence. The sequences of the S and L segments were identical. Two amino acid differences and one nucleotide difference in the 5' UTR were identified. The FLI sequence encodes lysines (K) at positions 746 and 1340, whereas the CVI sequence encodes glutamic acids (E) at these positions. It is possible the lysines present in the FLI sequence result from cell adaptation and that this could negatively influence virulence of the virus (i.e. attenuated virus). For this reason, the M genome segment from both FLI and instant isolates were synthesized.

Example 4 – Reverse genetics production of SBV

The full genome sequences corresponding to the L, S and M sequences (in *antigenomic* orientation; and flanked by a minimal T7 promoter sequence on the 5' end and a hepatitis- δ ribozyme sequence and T7 terminator sequence on the 3' end), were synthesized by the GenScript Corporation. The sequences were cloned into pUC57 plasmids, resulting in pUC57-SBV-S, pUC57-SBV-L and pUC57-SBV-M. Two versions of pUC57-SBV-M were developed, one encoding the FLI sequence (referred to as M-Germ) and one encoding the CVI sequence (referred to as M-Neth).

Rescue of the viruses from cDNA was performed by co-transfection of plasmids pUC57-SBV-S, pUC57-SBV-M (Neth or Germ) and pUC57-SBV-L into BSR-T7/5 cells using JetPEI as the transfection reagent. Rescue of SBV-Germ was relatively straightforward. Transfection of the plasmids resulted in cytopathic effect (CPE) on BSR-T7/5 cells. Initial passaging was performed on BSR-T7/5 cells, but this resulted in loss of the virus (similar results were obtained with passage of field-collected isolates on BHK cells). Vero cells were also inoculated with the rescued virus, which resulted in clear CPE (FIG. 2B). Immunoperoxidase monolayer assays (IPMA) with a convalescent sheep serum confirmed that this cpe was caused by SBV (FIG. 2D).

Rescue of the SBV-Neth virus required considerably more effort, which is possibly explained by the putative cell-adaptive mutations present in the SBV-Germ virus. P1 stocks (rescued on BSR-T7/5 cells and passaged once on Vero cells) of both viruses are now available. Stocks SBV-Germ: 106.61 TCID₅₀/ml; SBV-Neth: 107.39 TCID₅₀/ml. The full genome sequences including flanking sequences are depicted in FIGs. 3-5.

Example 5 – Efficacy of Reverse Genetics-produced SBV vaccine in Sheep

Objective. Demonstrate the efficacy of different doses of SBV vaccine by vaccination/challenge in sero-negative lambs. Efficacy will be tested after one dose of vaccine and compared to a group of non-vaccinated age-matched lambs.

Materials and Methods. Twenty conventional lambs, both males and females, less than 6 months of age at the time of vaccination. All lambs will be seronegative against SBV.

Vaccines. Three batches of vaccine containing a low (L), medium (M) or high (H) dose of inactivated SBV antigen, respectively in the presence of ALSAP adjuvant (aluminum hydroxide + saponin + TS6 adjuvant described in US7371395).

Randomization and vaccination. At reception, all lambs will be randomized based on body weight to 4 groups (G1- G4) containing 5 lambs each. On D0, lambs from group G1, G2 and G3 will receive one dose of vaccine L, M or H, respectively by SQ administration on the thorax. Prior to vaccination, the injection site will be shaved and disinfected. Lambs from G4 will not be vaccinated and serve as negative controls.

Challenge and follow-up. On D14, all lambs will be infected SQ with 1-3 ml of a suspension of a virulent strain of SBV. Lambs will be clinically examined (including rectal

temperatures) for 14 days after challenge and blood will be collected on days 1-7, 10 and 14 after challenge and tested for the presence of SBV (viremia) by q-PCR

Evaluation of results. Clinical signs and rectal temperatures will be described and discussed. Groups will be statistically compared to each other with respect to the amount of SBV detected in the blood following challenge expressed as the area under the curve.

* * * * *

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.

What is claimed is:

1. An immunological composition comprising a Schmallenberg virus (SBV) or portion thereof.
2. The composition of claim 1, wherein the virus comprises an S, M, and L segment.
3. The composition of claim 2, wherein the SBV comprises polynucleotides having at least 90% sequence identity to polynucleotides having the sequence as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).
4. The compositions of claim 2, wherein the SBV comprises a polynucleotide complementary to a polynucleotide having at least 90% sequence identity to the polynucleotide having the sequences as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).
5. The composition of claim 2, wherein the S segment encodes a peptide having at least 90% homology to SEQ ID NO:4; the M segment encodes a peptide having at least 90% homology to SEQ ID NO:10 or 11; and the L segment encodes a peptide having at least 90% homology to SEQ ID NO:15.
6. The composition of claim 3, wherein the S, M, and L segments encode peptides as set forth in SEQ ID NO:4, SEQ ID NOs:10 or 11, and SEQ ID NO:15, respectively.
7. The composition of claim 3, which is a vaccine composition, and further comprises a veterinarily acceptable carrier, saponin, and aluminum hydroxide.
8. A reverse genetics system for producing the vaccine composition of any one of claims 1 to 5.
9. The system of claim 8, comprising one or more plasmids comprising nucleotides having at least 70% homology to the sequence of SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).
10. The system of claim 9, comprising nucleotides having the sequences as set forth in SEQ ID NOs:3, 9, and 14.
11. The system of claim 8, comprising 3 separate plasmids, wherein a first plasmid encodes the S segment, a second plasmid encodes the M segment, and a third plasmid encodes the L segment.
12. The system of claim 11, wherein the S, M, and L segments encode peptides as set forth in SEQ ID NOs:4 (S segment), 10 or 11 (M segment), and 15 (L segment).

13. The system of claim 11, wherein the first plasmid encodes S segment 5' and 3' UTR, the second plasmid encodes the M segment 5' and 3' UTR, and the third plasmid encodes the L segment 5' and 3' UTR.
14. The system of claim 11, wherein the first plasmid comprises the sequences as set forth in SEQ ID NOs:2 & 3; the second plasmid comprises the sequences as set forth in SEQ ID NOs: 8 & 12 or 13; and the third plasmid comprises sequences as set forth in SEQ ID NOs:13 & 16.
15. The system of claim 11, where the plasmids comprise a T7 minimal promoter as set forth in SEQ ID NO:7 and a ribozyme / T7 terminator as set forth in SEQ ID NO:6.
16. A cDNA useful for the production of the virus of claim 1, comprising a nucleotide coding for a SBV S, M, or L segment.
17. The cDNA of claim 16, comprising nucleotides having at least 90% homology to the sequences as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).
18. An isolated RNA molecule transcribed from the cDNA of claim 16 or 17.
19. The RNA molecule of claim 18, comprising sequence complementary to SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).
20. A method for producing the SBV of claim 1, comprising the steps of:
 - a. producing a cDNA encoding each of the SBV segments (S, M, and L);
 - b. co-transfecting the cDNAs into cells capable of producing the SBV from said cDNAs; and
 - c. allowing the cells to make the virus, thereby producing the virus.
21. The method of claim 20, wherein the cDNAs have the sequence as set forth in SEQ ID NO:3 (S segment); SEQ ID NO:9 (M segment); and SEQ ID NO:14 (L segment).
22. The method of claim 20, wherein the cells are BSR-T7/5 cells.
23. A method for providing an animal protection against SBV comprising the steps of administering the composition of claim 1 or 2 to said animal, thereby providing the protection.
24. The method of claim 20, wherein the dose is administered IP or SC and in a dose range of about 10 µg to about 300 µg.
25. The method of claim 20 or 21, wherein the protection lasts for at least about 1 year.

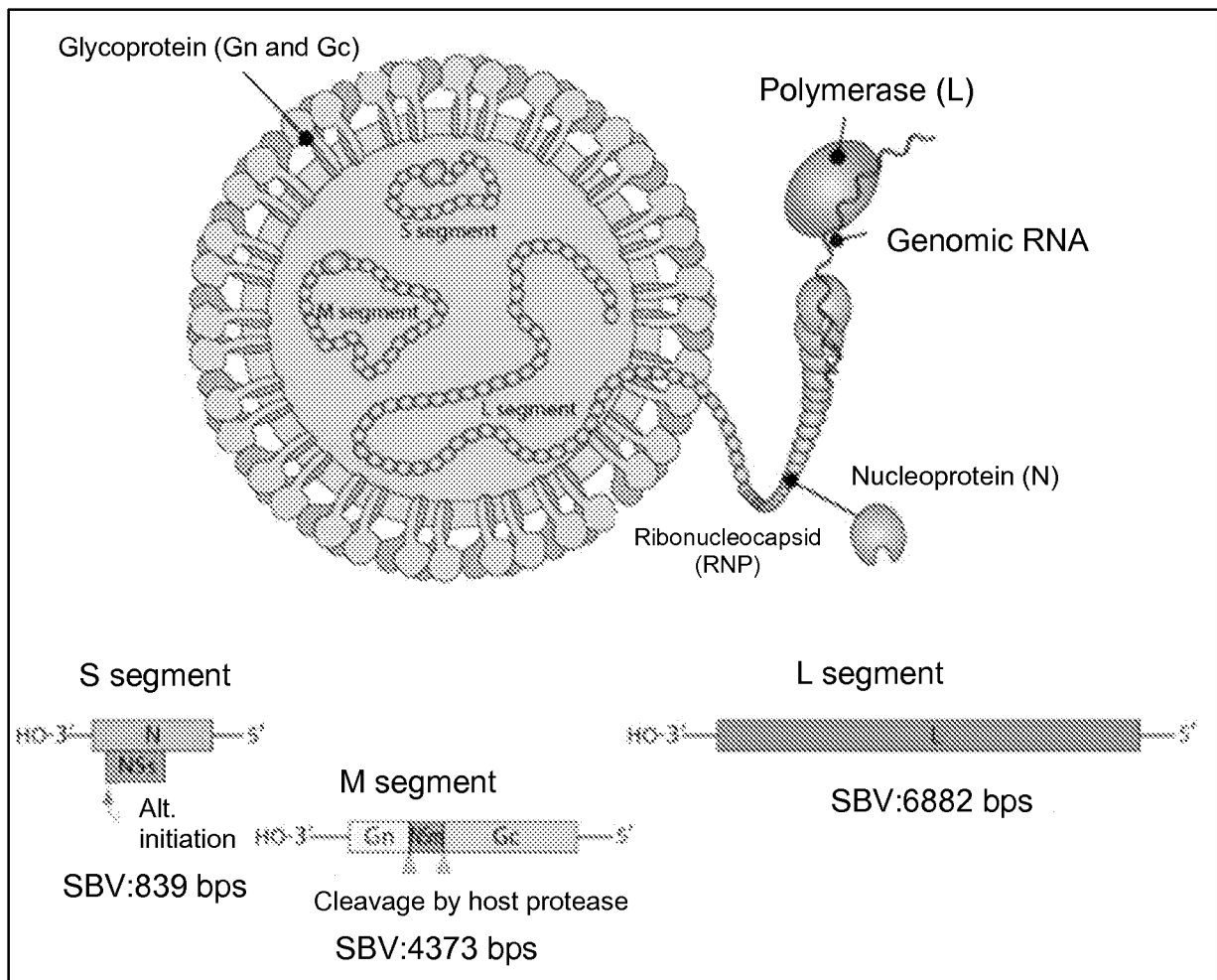
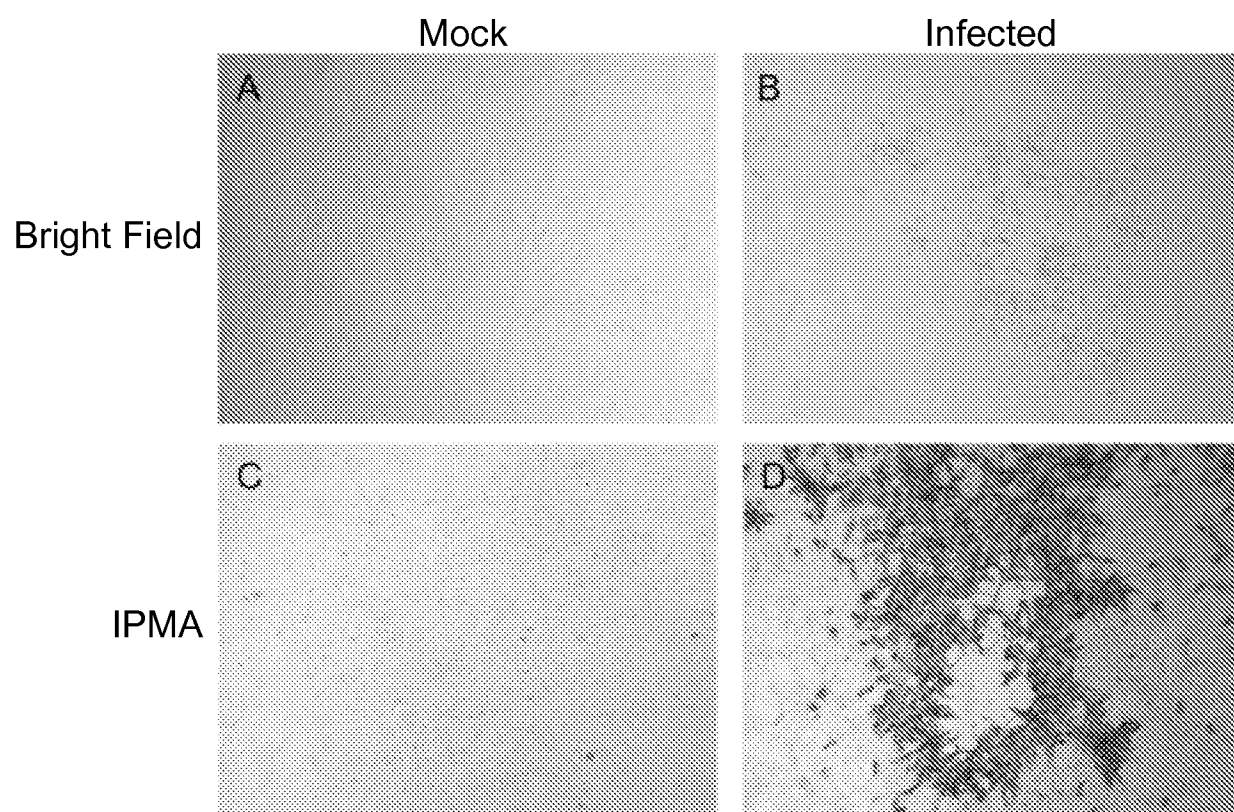


FIG. 1

*FIG. 2*

S segment +T7/Ribo/T7t:

SEQ ID NO:1 T7 minimal promotor
TAATACGACTCACTATAG

SEQ ID NO:2 ("S" 5' UTR)
AGTAGTGAAGTCCACTATTAACCTACAGAAAT

SEQ ID NO:3 ("S" ORF nucleotide)
ATGTCAAGCCAATTCATTTTTGAAGATGTACCACAACGGAATGCAGCTACATTTAACCCGGAGGTCGGGT
ATGTGGCATTATTATTGGTAAGTATGGGCAACAACCTCAACTTCGGTGTTGCTAGAGTCTTCTTCCTCAACCA
GAAGAAGGCCAAGATGGTCCTACATAAGACGGCACAACCAAGTGTGCGATCTTACTTTTGGTGGGGTCAAA
TTTACAGTGGTTAATAACCATTTTCCCCAATATGTCTCAAATCCTGTGCCAGACAATGCCATTACACTTC
ACAGGATGTCAGGATATCTAGCACGTTGGATTGCTGATACATGCAAGGCTAGTGTCCCTCAAACCTAGCTGA
AGCTAGTGCTCAGATTGTCATGCCCTTGTCTGAGGTTAAGGGATGCACCTGGGCCGATGGTTATACAATG
TATCTTGGATTTGCACCTGGGGCCGAAATGTTCTTGTATGCTTTTGAAGTCTTCTATCCACTAGTTATTGAAA
TGCATAGGGTCTCAAGGACAATATGGATGTAAATTTTATGAAAAAAGTCTCCGCCAACGCTATGGAAC
AATGACTGCTGAAGAATGGATGACTCAGAAAATAACAGAAATAAAAGCTGCTTTTAATTCTGTTGGACAG
CTTGCTGCGGCCAAATCTGGATTCTCTCCTGCTGCTAGAACCTTCTGCAGCAATTCCGGTATCAACATC

SEQ ID NO:4 ("S" ORF amino acid)
MSSQFIFEDVPQRNAATFNPEVG YVAFIGKYGQQLNFGVARVFFLNQKKAKMVLHKTAQPSVDLTFGGVK
FTVVNNHFPQYVSNPVPDNAITLHRMSGYLARWIADTCKASVLKLAESAQIVMPLAEVKGCTWADGYTM
YLGFAPGAEMFLDAFDYPLVIEMHRVLKDNMDVNFMKKVLQRQYGTMTAEWMTQKITEIKAAFNSVGQ
LAWAKSGFSPAARTFLQQFGINI

SEQ ID NO:5 ("S" 3' UTR)
TGAACCTCTTCATCACAGATCTTCAATTTCCGTGCAATATGTCTATGTATTGCACACCATTATACTGCAA
GGCTTCTGTTAAGATAGTTAATAAGTGGAGAACACTACT

SEQ ID NO:6 (Ribozyme/T7 Terminator)
GGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCATCCGAAGGAGGACGTCGTCCACTCGG
ATGGCTAAGGGAGAGCTCGGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACC
GCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTG

FIG. 3

Schmallenberg Virus M segment:

SEQ ID NO:7 (T7 minimal promotor)
TAATACGACTCACTATAG

SEQ ID NO:8 ("M" 5' UTR)
AGTAGTGAACCTACCACAATCAAA

SEQ ID NO:9 ("M" ORF nucleotide; "A/G's" are "A's" in SBV-Germ and "G's" in SBV-Neth)

ATGCTTCTCAACATTGTCTTGATATCTAACTTAGCCTGTTTAGCTTTTGCCTCCCACTTAAGGAAGGCA
CTAGAGGGTCTAGGTGCTTCCTGAATGGCGAACTGGTTAAACTGTTAACACATCAAAGGTCGTTTCAGA
ATGCTGTGTGAAAGACGACATATCTATCATTAAATCAAATGCTGAACATTATAAATCAGGAGATCGGTTG
GCTGCTGTAATAAAATATTATCGTTTATATCAGGTGAAGGATTGGCATTCTTGCAATCCAATTTATGATG
ACCACGGTTCCTTTATGATATTAGATATAGATAAATACTGGCACATTAATCCCTAAAATGCATACATGCAG
AGTTGAATGCGAAATAGCACTGAATAAAGATACTGGCGAAGTTATATTGAATTCATATCGAATTAACCAC
TACCGAATCTCGGGCACAATGCATGTATCAGGTTGGTTTAAAAACAAAATTGAGATTCTTTTGGAAAACA
CATGCGAATCCATTGAGGTAACATGTGGATTAAAAACACTTAATTTTCATGCATGTTTCCATACCCATAA
GTCATGCACCCGCTATTTTAAAGGATCAATCCTGCCGGAATTGATGATCGAATCATTTTGTACGAATCTT
GAATTAATACTGCTAGTAACCTTCATATTAGTTGGGTCTGTCTATGATGATGATATTGACGAAAACATATA
TAGTATATGTGTTTCATTCCCTATATTTTATCCATTTGTGAAATTATATGCTTATATGTACAACAAATATTT
TAAATTGTGTAAAAATTGCCTGTTAGCAGTACATCCCTTTACAAATTGCCCATCGACATGCATCTGTGGA
ATGATTTTACTACTACCACTGAATCACTCAAATTGCATCGCATGTGTAACAATTGTTCTGGCTATAAAGCAT
TGCCGAAAACAAGGAAATTGTGTAAAAGTAAAATATCCAATATAGTGCTATGTGTGATAACATCACTGAT
ATTTTTCTCATTTATCACACCTATATCGAGTCAATGTATCGATATAGAAAACTGCCAGACGAGTATATT
ACATGTAAAAGAGAGCTAGCTAATATCAAAGCTTGACAATTGATGACACATATAGCTTTATATATTCTT
GTACATGCATAATTGTGTTAATATTACTTAAAAAGGCAGCAAAGTATATCTTGTACTGCAACTGCAGCTT
TTGTGGTATGGTACATGAACGACGTGGATTGAAGATAATGGACAACCTTTACAAACAAGTGCCTAAGTTGT
GTATGCGCAGAAAACAAGGGCTTAACAATTCACAGAGCCTCTGAGAAATGTCTGTTCAAATTTGAATCAA
GTTATAATAGGACCGGGTTGATAATCTTTATGCTTCTGTTAGTCCCAACAATTGTAATGACGCAAGAAAC
TAGTATTAATGCAAAAACATTCAATCAACTCAGCTTACAATAGAGCACCTGAGTAAGTGCATGGCATT
TATCAAAAATAAAACAAGCTCACCAGTTGTAATCAATGAAATAATTTAGATGCTTCAGTAGACGAACAAG
AATTAATAAAAAGTTTTAACTTGAAGTGAATGTCATAGATAGGTTTATTTCCGAATCTAGTGTTATTGA
GACTCAAGTTTATTATGAGTATATAAAATCACAGTTGTGCCCTCTCCAAGTGCATGATATTTTCACTATC
AATTCAGCAAGTAACATACAATGGAAAGCACTGGCCCGAAGTTTCACCTTAGGAGTGTGCAATACGAATC
CTCATAAACATATATGTAGATGCTTGGAGTCTATGCAAATGTGCACATCAACCAAGACAGACCACGCTAG
GGAAATGTCAATATATATGATGGTCAATCAGATCGCTTTGAGCATGACATGAAAATAATATTGAATATA
ATGAGATATATAGTCCCTGGATTAGGTGAGTCTTGCTTGATCAAATCAAACAAACAAAAGACTACCAAG
CTTTACGCCACATACAAGGTAAGCTTTCTCCTAAATCGCAGTCAAATTTACAACCTTAAAGGATTTCTGGA
ATTTGTTGATTTTATCCTTGGTGCAACGTGACAATAGAAAAAACCCCTCAAACATTAATACTACATTATCT
TTGATAAAAGGAGCCCACAGAACTTGGATCAAAAAGATCCAGGTCCAACACCAATACTGGTATGCAAAT
CACCACAAAAGTGGTATGCTACTCACCACGTGGTGTACACACCCAGGAGATTATATATCATGC (**A/G**)
AATCTAAGATGTATAAGTGGCCATCTTTAGGGGTATACAAACATAATAGAGACCAGCAACAAGCCTGCAG
CAGTGACACACATTGCCTAGAGATGTTTGAACCAGCAGAAAGAACAATAACTACAAAATATGCAAAGTA
AGTGATATGACTTATTCAGAATCGCCATATAGTACTGGAATACCATCATGCAACGTGAAGAGATTTGGAT
CATGTAATGTAAAGGGGTCATCAATGGCAAATTGCAGAATGCTCAAATGGCTTATTTTACTATGTTTCAGC
TAAAGCCCATTCGAAAACCTAACGATATAACACTGTACTGTTTATCAGCAAATTGCCTGGACTTGCGTTAT
GCAT

FIG. 4 (1/3)

TCAGATCCAGTAGTTGTTTCAGATATAGTATGGGATACAAGTTATCGAAATAAAATTAACACCTAAATCTAT
 TAATCATCCAGATATTGAAAACCTACATAGCAGCGCTTCAGTCAGATATTGCAAATGATTTAACTATGCAC
 TACTTTAAACCATTAAAAAACCTTCCAGCAATAATTCCTCAATACAAAACAATGACATTGAATGGGGACA
 AGGTATCAAATGGTATTAGAAATAGTTATATCGAGTCGCACATCCCTGCAATTAATGGTTTATCAGCAGG
 GATTAATATTGCCATGCCAAATGGAGAAAGCCTCTTTTCCATTATTATCTATGTCAGAAGAGTAATAAAT
 AAAGCATCGTATCGATTTCTATATGAAACAGGACCCACAATTGGAATAAATGCCAAGCACGAAGAGGTAT
 GTACCGGGAAGTGCCCAAGCCCAATACCACATCAAGATGGTTGGGTCACATTCTCAAAGGAAAGATCAAG
 TAATTGGGGCTGTGAAGAATGGGGTTGCTTGGCAATAAATGATGGTTGTTTATATGGGTCATGTCAAGAC
 ATAATAAGGCCTGAATATAAGATATACAAGAAGTCTAGTATTGAACAAAAGGATGTTGAAGTTTGTATAA
 CCATGGCCCATGAATCATTCTGCAGTACCGTTGATGTTCTCCAACCTTTAATTAGCGACAGGATACAATT
 AGATATCCAAACGATTCAAATGGACTCTATGCCAAATATAATTGCAGTCAAGAATGGGAAAGTTTATGTT
 GGAGATATCAATGACTTAGGTTTCGACAGCAAAGAAATGTGGCTCAGTCCAATTATATTCTGAAGGGATCA
 TTGGATCGGGAACCCCAAAATTTGATTATGTTTGGCATGCATTCAATCGTAAAGATGTCATCCTTCGAAG
 ATGCTTTGATAACTCATATCAGTCTTGTCTTCTTGGAAACAAGATAATACATTAAGTATTGCTTCTACC
 AGTCATATGGAAGTGCATAAAAAAGTTTCAAGCGTGGGTACAATCAATTATAAAATTATGTTAGGGGATT
 TTGACTACAATGCATATTCAACACAAGCAACAGTCACAATAGATGAGATCAGGTGTGGTGGTTGTTATGG
 CTGCCCTGAAGGAATGGCTTGCACACTCAAATTGAGTACCAATACCATCGGGAGTTGTTCAATAAAAAAGT
 AACTGCGATACATACATTAAATAATAGCAGTCGATCCGATGCAGAGCGAGTATTCATTAAAGTTAAACT
 GCCCCTAGCAACAGAGACAGTTTCAGTAAGTGTGTGCTCAGCTTCTGCTTACACAAAACCTTCAATATC
 TAAAAATCAACCAAAAATTGTTTTGAATTCCTTAGATGAAACATCTTACATCGAGCAACATGATAAAAAG
 TGTTCTACATGGCTTTCGAGAGTTTAT **(A/G)** AAGAAGGGATTAGCGTAATATTTTCAGCCTCTATTTGGC
 AACCTATCTTTCTATTGGAGACTGACAATATATATAATAATCTCTTTGATTATGCTAATTCTGTTTCTAT
 ACATATTAATACCACTGTGCAAACGGCTAAAAGGTTTATTGGAATACAATGAGAGAATATACCAAATGGA
 AAATAAATTTAAG

SEQ ID NO:10 (SBV-Germ sequence → wherein both variable nucleotides of
 SEQ ID NO:9 are "A")

MLLNIVLISNLACLAFLPLKEGTRGSRCLNGLVKTVNTSKVVSECCVKDDISIIKSNAEHYKSGDRL
 AAVIKYYRLYQVKDWHSCNPIYDDHGSFMILDIDNTGTLIPKMHTCRVECEIALNKDTGEVILNSYRINH
 YRISGTMHVSGWFKNKIEIPLNTCESIEVTCGLKTLNFHACFHTHKSCTRYFKGSILPELMIESFCTNL
 ELILLVTFILVGSVMMILTKTYIVYVFIPIFYFPVKLYAYMYNKYFKLCKNCLLAVHPFTNCPSTCICG
 MIYTTTESLKLHRMCNNCSGYKALPKTRKLCKSKISNIVLCVITSLIFFSFITPISSQCIDIEKLPDEYI
 TCKRELANIKSLTIDDTYSFIYSCTCIIVLILLKKAAYIILYCNCSCGMVHERRGLKIMDNFTNKCLSC
 VCAENKGLTIHRASEKCLFKFESSYNRTGLIIFMLLLVPTIVMTQETSINCKNIQSTQLTIEHLSKCMF
 YQNKTSPPVINEIISDASVDEQELIKSLNLNCNVIDRFISESSVIETQVYYEYIKSQLCPLQVHDIFTI
 NSASNIQWKALARSFTLGVCNTNPHKHICRCLESMQMCTSTKTDHAREMSIYYDGHDPDRFEHDMKIIILNI
 MRYIVPGLGRVLLDQIKQTKDYQALRHIQGLSPKQSNNLQLKGFLEFVDFILGANVTIEKTPQTLTTL
 LIKGAHRNLDQKDPGPTPILVCKSPQKVCYSRPGVTHPGDYISCKSKMYKWPSLGVYKHNRDQQQACSS
 DTHCLEMFEPARTITTKICKVSDMTYSESPYSTGIPSCNVKRFGSCNVRGHQWQIAECSNGLFYYVSAK
 AHSKTNDITLYCLSANCLDLRYAFRSSSCSDIWDTSYRNKLTTPKSINHPDIENYIAALQSDIANDLTMH
 YFKPLKNLPAIIPQYKMTLNGDKVSNIRNSYIESHIPAINGLSAGINIAMPNGESLFSIIIIYVRRVIN
 KASYRFLYETGPTIGINAKHEEVCTGKCPSPIPHQDGVWTFSEKERSNNGWCEEWGCLAINDGCLYGSCQD
 IIRPEYKIYKKSSIEQKDVEVCITMAHESFCSTVDVLQPLISDRIQLDIQTIQMDSMPNIIAVKNGKVYV
 GDINDLGSTAKKCGSVQLYSEGIIGSGTPKFDYVCHAFNRKDVILRRCFDINSYQSCLLLEQDNTLTIAST
 SHMEVHKVSSVGTINYKIMLGDFDYNAYSTQATVTIDEIRCGGCYGCPEGMACALKLSTNTIGSCSIKS
 NCDTYIKIIAVDPMQSEYSIKLNCPLATETVSVSVCSASAYTKPSISKQPKIVLNSLDETSYIEQHDKK
 CSTWLCRVYKEGISVIFQPLFGNLSFYWRLTIYIIISLIMLILFLYILIPLCKRLKGLLEYNERIYQMEN
 KFK

FIG. 4 (2/3)

SEQ ID NO:11 (SBV-Neth sequence → wherein both variable nucleotides of SEQ ID NO:9 are "G")

MLLNIVLISNLACLAFAFALPLKEGTRGSRCLNGELVKTVNTSKVVSECCVKDDISIIKSNAEHYKSGDRL
AAVIKYYRLYQVKDWHSCNPIYDDHGSFMILDIDNTGTLPKMHTCRVECEIALNKDTGEVILNSYRINH
YRISGTMHVSGWFKNKIEIPLNTCESIEVTCGLKTLNFHACFHTHKSCTRYFKGSILPELMIESFCTNL
ELILLVTFILVGSVMMMLTKTYIVYVFIPIFYFPVKLYAYMYNKYFKLCKNCLLAVHPFTNCPSTCICG
MIYTTTESLKLHRMCNCSGYKALPKTRKLCKSKISNIVLCVITSLIFFSFITPISSQCIDIEKLPDEYI
TCKRELANIKSLTIDDTYSFIYSCTCIIVLILLKKAAYIILYCNCSCGMVHERRGLKIMDNFTNKCLSC
VCAENKGLTIHRASEKCLFKFESSYNRTGLIIFMLLLVPTIVMTQETSINCKNIQSTQLTIEHLSKCMFAF
YQNKTSPPVINEIISDASVDEQELIKSLNLCNVIDRFIGESSVIETQVYYEYIKSQLCPLQVHDIFTI
NSASNIQWKALARSFTLGVCNTNPHKHICRCLESMQMCTSTKTDHAREMSIYYDGHDPDRFEHDMKIIILNI
MRYIVPGLGRVLLDQIKQTKDYQALRHIQGLSPKSQSNLQKGFLEFVDFILGANVTIEKTPQTLTTLTSL
LIKGAHRNLDQKDPGPTPILVCKSPQKVVCYSPRGVTHPGDYISCESKMYKWPSLGVYKHNRDQQQACSS
DTHCLEMFEPARTITTKICKVSDMTYSESPYSTGIPSCNVKRFSGSCNVRGHQWQIAECSNGLFYVYVSAK
AHSKTNDITLYCLSANCLDLRYAFRSSSCSDIVWDTSYRNKLTPKSINHPDIENYIAALQSDIANDLTMH
YFKPLKNLPAIIPQYKTMTLNGDKVSNNGIRNSYIESHIPAINGLSAGINIAMPNGESLFSIIIVRRVIN
KASYRFLYETGPTIGINAKHEEVCTGKCPSPIPHQDGVWTFSEKERSNWNWGCCEEWGCLAINDGCLYGSCQD
IIRPEYKIYKKSIEQKDVEVCITMAHESFCSTVDVLQPLISDRIQLDIQTIQMDSPNIIAVKNGKVYV
GDINDLGSTAKKCGSVQLYSEGIIGSGTPKFDYVCHAFNRKDVILRRCFDNSYQSCLLLEQDNTLTIAST
SHMEVHKVSSVGTINYKIMLGDFDYNAYSTQATVTIDEIRCGGCYGCPEGMACALKLSTNTIGSCSIKS
NCDTYIKIIAVDPMQSEYSIKLNCPLATETVSVSVCSASAYTKPSISKNPQKIVLNSLDETSYIEQHDKK
CSTWLCRVYEEGISVIFQPLFGNLSFYWRLTIYIIISLIMLILFLYILIPCKRLKGLLEYNERIYQMEN
KFK

SEQ ID NO:12 (SBV-Germ "M" 3' UTR)

TGATAAGCCTTATAACAATGAGCAATTATAAATGAATAAATAAAAAACAATAAAAAGATAAAACAAATAACAA
CATATATATGTGGTTACACATATATATGTAATTATTCAGCTGAGAAGTTTTTCATGTGGTAGAACAACACTAC
T

SEQ ID NO:13 (SBV-Neth "M" 3' UTR)

TGATAAGCCCTATAACAATGAGCAATTATAAATGAATAAATAAAAAACAATAAAAAGATAAAACAAATAACAA
CATATATATGTGGTTACACATATATATGTAATTATTCAGCTGAGAAGTTTTTCATGTGGTAGAACAACACTAC
T

SEQ ID NO:6 (Ribozyme/T7 Terminator)

GGGTCCGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCATCCGAAGGAGGACGTGCTCCACTCGG
ATGGCTAAGGGAGAGCTCGGATCCGGCTGCTAACAAAGCCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACC
GCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTG

FIG. 4 (3/3)

Schmallenberg Virus L segment:

SEQ ID NO:7 (T7 minimal promotor)
TAATACGACTCACTATAG

SEQ ID NO:13 ("L" 5' UTR)
AGTAGTGTACCCCTAATTACAATCACT

SEQ ID NO:14 ("L" ORF nucleotide)
ATGGAGACATACAAGATTAACATTTTTAGAGATAGGATCAACCAGTGTCTGAAGTGCTGAAGAAGCCAAAG
ACATTGTTGCTGATCTTCTCATGGCTAGACATGACTACTTTGGTAGAGAGGTATGTTATTACCTGGATAT
CGAATTCCGGCAGGATGTTCCAGCTTACGACATACTTCTTGAATTTCTGCCAGCTGGCACTGCTTTCAAC
ATTCGCAATTGTACACCAGACAATTTTATCATTACAAATGGCAAGCTTTATATCATTGACTATAAAGTAT
CAACTGATCATGCATATGGTCAAAAACTTATGAAAAGTACACCCAGATCTTTGGAGACGCATTGTCAGA
ATTGCCGTTTGATTTTGAAGTTGTGATCATCCGTGCTGACCCTCTGCGAGATACTATCCATGTTAATTCA
AATCAATTCTTGAAAAATTTTGGGCCGCTCAACATAAACCTTGATTTTACTTGGTTCTTTAATTTGCGAT
CCCTGATATATGAGAAATATAAGGATGACGACAGATTCCCTAGAAATTGTGAATCAAGGTGAATTTACGAT
GACTGGACCCTGGATTGATGAGGATACCCCGGAGCTCTATTACACCCTGTCTTTTGGAAATCTATGAT
TCTTTAGATGAGATGGCTAAACTGACATTCCATGAGTCTATGACATTTGATGCAACTCGCGGTGAGAAAT
GGAATCAAAATCTACAAAAGGTTATAAATAGATATGGCAATGATTATAACATTTTTGTGAAAGAGGCCGC
TGCAGGAATCTTTAGATGTGAAGGGAACACCCAAAACCAAATCATGATGAAATCACAATCGGTTGGAAT
CAAATGGTTCAAAGAGTGAGTACTGAGAGAAACCTGACTCAAGATGTCAGCAAGCAAAAACCATCTATTC
ATTTCATATGGGGTCAACCTGACGAAACATCAAATGCGACAACACCAAACTAATCAAGATTGCAAAAGC
ACTCCAAAATATTTCTGGCGAGTCTACATATATAAGCGCATTGAGAGCATTGGGTATGCTTATGGACTTT
TCTGAGAACACAGCTTTATATGAAGCACACACTAGCAAATAAAAAGTATGGCAAGACAGACATCGAAAA
GAATTGATACTAACTGGAACCAATCAAATAGGCACGGCGACAATTTATTGGGAACAGCAGTTTAACT
GGATACTGAAATAATGAATACAAAAGACAAATCACATTTGCTAAAAGATTTTCTTGGCATAGGGGGTAC
GTGCAATTTTCAAAAAGACCATTGACGATTTGGATACTGACAAACCTACTATATTAGATTTCAACAAAA
AGGAAGTCATTGATTTTGCAAATTCAGTATGAAAATGTAAAGAAAATACTATCCGGAGATAATAATCT
AGAGCGTATAGGATGTTATTTAGAAGAATATGGTGCAATATTGCATCATGTTCAAAGGATACATGGGAT
CAGATTAACCAGATAGGGAAGTCAAATTACTGGGCTTGTATTAAGATTTTTCAGTCTTGATGAAAAATA
TGTTGGCAGTTTCTCAATATAATAGGCACAATACTTTTTCGTGTAGTGTGTTGTGCAACAATAATCTGTT
TGGGTTTGTAAATGCCTTCTTCTGATATTAAAGCAAAGCGATCCACACTTGTTTACTTCTTAGCTGTGTTG
CATTCTACTCCTCAGAATGTGATGCACCACGGTGCATTGCATGCGACATTTAAAACCTGGTTCAAATACC
TTAGTATCTCTAAAGGAATGCGTTTAGATAAAGAACGATGTCAACGCATAGTTAGTTCACCGGGACTTTT
TATGTTGACTACATTGATGTTTGCAGGAGACAATCCGACACTCAATTTGACTGATGTCATGAATTTTACA
TTCCACACTTCCCTGTCTATAACCAAAGCTATGCTGTCATTGACAGAACCATCAAGATATATGATAATGA
ATTCAATTAGCCATATCCAGTCATGTTAGAGATTATATAGCAGAAAAATTTGGCCCTTATACAAAGACCAG
CTTCTCTGTAGTAATGGCAAACCTGATTTAAAGGGGATGTTATATGGCATATAATCAAAGAGATAAAGTA
GACATGAGGAATATCTGCCTAACAGATTATGAAATAACTCAAAAAGGTGTGAGAGATAACAGAGACCTAT
CATCAATCTGGTTTGAAGGCTATGTATCACTAAAAGAATATATTAACCAAATATATCTACCATTTTACTT
CAATTCAAAAGGTTTGCATGAAAAGCATCATGTTATGATAGATCTGGCTAAGACAATCTTAGATATAGAA
AGGGACCAGAGATTAAATATCCCAGGAATATGGTCTACAACACCTAGAAAACAACTGCAAAATTTAAATA
TAACTATCTATGCAGTTGCAAAAAATCTAATAATGGACACTGCTAGACATAATTATATTAGATCACGGAT
AGAAAACACAAACAACTTAAATAGATCGATATGCACTATTTCTACATTCACCAGCTCTAAATCATGTATT
AAAGTAGGCGACTTTGAGAAAGAAAAAAGCTCAGCAACAAAAAAGGCTGCAGATTGCATGTCAAAAGAGA
TAAAGAAGTATACAATTGCAAACCCAGAATTTGTTGATGAAGAGTTACTAAATGCAACTATAAGACATTC

FIG. 5 (1/4)

ACGCTATGAAGACTTAAAAAAGCAATCCCGAATTATATTGACATTATGTCAACTAAAGTATTTGATTCT
CTGTACCAGAAAATAAAAAGGAAGGAGATAGATGATAAACCCACTGTGTATCATATACTCTCTGCTATGA
AGAATCACACAGATTTTAAAGTTTACATTCTTTAAACAAAGGCCAAAAAACAGCAAAGGATAGGGAAATATT
CGTAGGCGAATTTGAGGCAAAATGTGCTTGTATTTAGTGGAGAGGATATCTAAAGAACGCTGTAAAGTTG
AATCCAGATGAGATGATTAGTGAACCAGGCGATTCTAAATTGAAAAAATTAGAAGAGCTTGCAGAGTCTG
AAATACGATTCACAGCAGCAACTATGAAACAGATCAAAGAACGCTATTTAGCAGAAATGGGAGAAGCAAG
CCATATGATCGCATATAAACCACATTCTGTAAAGATTGAAATCAATGCAGACATGTCAAAATGGAGTGCC
CAAGATGTTTTATTCAAATATTTCTGGTTGTTTGCATTAGATCCCGCACTTTATCTGCAAGAAAAAGAAA
GGATATTGTACTTCCTATGCAATTATATGCAAAAAAAGCTAATTCTGCCTGATGAAATGCTCTGTAGCAT
CCTTGACCAACGTATCAAACATGAGGATGATATAATATATGAAATGACCAATGGCTTATCGCAAAATTGG
GTCAATATTAAACGGAACGGCTGCAGGGGAATCTCAATTACACAAGTAGCTACCTACATTCATGTTCTA
TGAATGTTTATAAGGATATTCTAAAGAGAGCAGCCACTTTACTAGAAGGGGAAGTTTTAGTCAATTCTAT
GGTTCATTCTGATGACAATCACACTTCAATAGTGATGATCCAAGATAAATTAGATGATGATATTGTTATT
GAATTTTCTGCAAACTATTTGAAAAAATATGTCTAACTTTTGGAATCAAGCAAATATGAAGAAGACAT
ATATAACAAATTTCATAAAGGAGTTCGTTTCACTTTTTAATATTTATGGTGAGCCATTTTCTGTTTTATGG
TCGCTTTATTTTGACATCTGTTGGCGATTGTGCTTTTCTTGGACCATATGAGGATGTTGCCAGTAGGTTG
TCTGCAACGCAGACAGCAATTAAGCATGGAGCACCTCCATCACTTGCATGGACTGCTATTGCATTAACCTC
AGTGGATAACACATAGCACATATAACATGCTTCCAGGTCAAATCAATGATCCTACTTCATCTTTACCTAG
TCATGATAGATTTGAGCTGCCTATAGAATTGTGTGGCTTAATAAATTCAGAATTACCCACTATAGCTATA
GCAGGTTTGAAGCAGATAATCTAAGTTATTTAGTTAGGTTATCAAAAAGAATGTCCCTATACATCTTT
GCCGTGAACCAATCCAGCATCAATATGAGAATATACATACATGGGATATAAGTAACTGACACAATGTGA
TATTTTCAGACTTAAGCTTTTAAGATACATGACGTTAGACTCAACTATGTCATCTGATGATGGAATGGGC
GAACTAGTGAAATGAGATCTAGGTCTCTTCTGACACCAAGAAAATTCACTACTGCAAGTTCGTTATCTA
GATTGCATTCATATGCTGATTATCAAAAACAATAACAAGACCAACAGAAAATTGAAGAATTATTTGAATA
TTTTATAGCCAACCCTCACTATTGGTTACAAAAGGTGAGACTTGTGAAGAGTCTGTATGTCTGTATTG
TTCAGATACAACAGTCGTAAATTTAAAGAATCATTGTCTATTCAAACCCAGCTCAGCTCTTCATAGAAC
AAGTATTGTTTGCAATAAACCAATGATAGACTATACAAGTATTCATGATAGGTTGTTTGGTATACAAGA
TGACCCAAATATAAATGATGCTACATGTATTATTGGCAAGAAGACTTTTGTTGAAACATATCAGCAAATA
AAAATTGATGTAGAAAAATTTACACTTGATGTAGAGGATATAAAGACGATATATAGCTTCTGTATAATGA
ACGACCTATATTAGTTGCTTGTGCAACAACCTTGTTAATTTCAATACAGGGAGTGGAGATGCAACGATT
GGGTATGACATGCTGTTATATGCCGGAGATTAAGAGCCTTAAAGTAATTTATCATAGTCCTGCTCTCGTA
TTACGTGCTTATGTAACAGATAACTATGAGCAAAAAGGGATGGAGCCAGATGAAATGCGGAGAGATATAT
ATCATTTAGAAGAATTTATAGAGAAGACAAAATTGAGGACAAATATGCAAGGGAGAATTGCAAATAATGA
AATTAAGTTAATGAAGCGAGATTTGAAATTTGAAGTGCAGGAATTGACTAAATCTATCAGATCTGTTAT
GAATATGTGAAATCAACAGAACACAAAATTTAAATATTCATCCTTCCAAAAAAGGCTTACACTCCCATTG
ATTTCTGCTCATTAGTAACAGGTAATCTGATATCAGACAACAAATGGATGGTTGTTCACTATTTAAAACA
AATAACTGTCCCAGCAAAGAAGGCACAAATAGCCACATCTATAGATCTGGAAATACAAATAGCCTACGAA
TGTTTCAGGCTAATTGCACATTTTGTGATATGTTCCCTAAATGATGACTCCAAAAAAGCTTATATTAATG
CAATTATTAACACATATACATACAAGGATGTTCAAGTATCCAGTCTCTACAAGAAAATCAAAAATTCGAG
ACTACGTTCAAAAATTATACCATTATTATATCACCTGGGCGATTTGCAACAAATAGACGTTGACAGATTT
GATGCAGAAAAAGCAGAAGAGCAGATCACATGGAATAACTGGCAAACATCTCGAGAATTTACTACTGGTC
CAATTGATCTATCAATCAAAGGTTATGGACGGTCAATAAGGATCGTAGGTGAGGACAACAAGCTTACAGC
TGCAGAAATGCAATTGTCAAGAGTGAGAAGTGATATAGTATCAAGGCATGGACAGGCTTTATTGAACAAA
CCTCATGGGCTAAAATTAGAGAAAAATGGAACCAGTGACTGATCTAAATCCTAAATTATGGTATATTGCAT
ACCAATTGCGTGAGAAAAAGCGGTATCACTATGGGGTCTTTAGTACATCTTATATAGAAGAGCATAACTC
AAGGATAGAAGCATCTCGGATACGTAAGACTAATAAATGGATACCAGTTTGCCCTATTGCTATATCAAAA

FIG. 5 (2/4)

CAATCATCTGATGGAAAGCCTAGTCTTGCAAAAATCCCTATGTTAAATATTGGGGAGATTAAATTTACAA
 AACTACAGATTGCAGTAGATGATCATGCAATGATTAGGAAAGCCCCATTTAGTAAGATGGTGTCTTTGA
 TGGCCACCCATACAGAGCGGTGGCATTGACATTGGAAAGCTTATGAAGAACCAAAATATTCTCAATTTG
 AGGTTAGATAATATACAGAGTATAACATTATTAGATTTGTGCCGCATATTTTCATGCCGAGGGTCTAAAG
 TGGATCAAGATGCATTTGAATTCTTATCTGATGAACCTTTGGATGAAGATGTTATTGATGAATTAGATAG
 CTCACCTGCATTAGTGGTATCTTACACAAAGAAATCAACCAATCCAATAGTTTCAAAAATGTTATAGTT
 AGAGCATTGATAAGAGAATGTGATATATTTGAAGATATAATGGACATAACAGACGATGGATTCACATCTG
 ATAGCAATCTAGAGGTGTTAGAAAACCTTAACATGGATTTTAAATATGCTCGCAACAAATCAGTGGTCTAC
 AGAACTGTTAGCATGCATACACATGTGTTTATATCGCAATGAGATGGATCATATCTATCACAATTTTCAA
 GTTCCAGAAATATTTGTGCGACAATCCAATCTCATTAAATGTAAAGTGGGATGAAGTAATTATGTTCTTAA
 ACATACTGCGAGACAGAGATTACAAATTTGAGCCATGGGTGTCTATACTGAATCATTCCTTAATAAGC
 TATAGAGTATGCTTACAAAAGATGGAAGAGGAGAGGAAGCAGAAATCAACAGGCATCAACAAATCTTA
 AAGGGTAAAAAATGGGTGGCAGATCAAAGTTTGATTTCCAG

SEQ ID NO:15 ("L" ORF amino acid)

METYKINIFRDRINQCRSAEEAKDIVADLLMARHDYFGREVCYYLDIEFRQDVPAYDILLEFLPAGTAFN
 IRNCTPDNFIIHNGKLYIIDYKVSTDHAYGQKTYEKYQIFGDALSELFPDFEVVIRADPLRDTIHVNS
 NQFLEIFGPLNINLDFTWFFNLRLSLIYEKYKDDDRFLEIVNQGEFTMTGPWIDEDTPELYSHPVFLEFYD
 SLDEMAKLTFHESMTFDATRGEKWNQNLQKVINRYGNDYNI FVKEAAAGIFRCEGNYPKPNHDEITIGWN
 QMVQRVSTERNLTQDVSKQKPSIHFIWGQPDETSNAATPKLIKIAKALQNISGESTYISAFRALGMLMDF
 SENTALYEAHTSKLKSMAQTSKRIDTKLEPIKIGTATIIYWEQQFKLDTEIMNTKDKSHLLKDFLGIGGH
 VQFSKKTIDDLDTDKPTILDFNKKEVIDFCKFQYENVKKILSGDNNLERIGCYLEEYGANIASCSKDTWD
 QINQIGKSNIYACIKDFSVMKNMLAVSQYNRHNTFRVCCANNLFGFVMPSSDIKAKRSTLVYFLAVL
 HSTPQNMVHHGALHATFKTGSKYLSISKGMRLDKERCQRIVSSPGLFMLTTLMFAGDNPTLNLTDVMNFT
 FHTSLSITKAMLSLTEPSRYMIMNSLAISSHVRDYIAEKFGPYTKTSFSVVMANLIKRCGYMAYNQDKV
 DMRNICLTDYEITQKGVDRNLDLSSIWFEQYVSLKEYINQIYLPFYFNSKGLHEKHHVMIDLAKTILDIE
 RDQRLNIPGIWSTTPRKQTANLNITIIYAVAKNLMIDTARHNYIRSRIENTNNLNRSICTISTFTSSKSCI
 KVGDFEKEKSSATKKAADCMSKEIKKYTIANPEFVDEELLNATIRHSRYEDLKKAIPNYIDIMSTKVFDS
 LYQKIKRKEIDDKPTVYHILSAMKNHTDFKFTFFNKGQKTAKDREIFVGEFEAKMCLYLVERISKERCKL
 NPDEMISEPGDSKLKLEELAASEIRFTAATMKQIKERYLAEMGEASHMIAKPHSVKIEINADMSKWSA
 QDVLFKYFWLFALDPALYLQEKERILYFLCNMQKKLILPDEMILCSILDQRIKHEDDIYEMTNGLSQNW
 VNIKRNWLQGNLNYTSSYLHSCSMNVYKDILKRAATLLEGEVLVNSMVHSDDNHTSIVMIQDKLDDDIVI
 EFSAKLFEKICLTFGNQANMKKTYITNFIKEFVSLFNIYGEPPSVYGRFILTSGDCAFLGPYEDVASRL
 SATQTAIKHGAPPSLAWTAIALTQWITHTSTYNMLPGQINDPTSSLPSHDFELPIELCGLINSELPTIAI
 AGLEADNLSYLVRLSKRMSPHLCREPIQHQYENIHTWDISKLTQCDIFRLKLLRYMTLDSTMSSDDGMG
 ETSEMRSRSLTPRKFTTASSLSRLHSYADYQKTIQDQKQIEELFEYFIANPQLLVTKGETCEEFCMSVL
 FRYNSRKFKESLSIQNPAQLFIEQVLFANKPMIDYTSIHDLRFGIQDDPNINDATCIIIGKKTFFVETYQQI
 KIDVEKFTLDVEDIKTIYSFCIMNDPILVACANNLLISIQGVEMQRLGMTCCYMPEIKSLKVIYHSPALV
 LRAYVTDNYEQKGMEPDEMRRDIYHLEEFIEKTKLRNMQGRIANNEIKLMKRDLKFEVQELTKFYQICY
 EYVKSTEHKIKIFILPKKAYTPIDFCSLVTGNLISDNKWMVVHYLKQITVPAKKAQIATSIDLEIQIAYE
 CFRLIAHFADMFLNDDSKAYINAIINTYTYKDVQVSSLYKKIKNSRLRSKIIPLLYHLGDLQQIDVDRF
 DAEKAEQITWNNWQTSREFTTGPIDLSIKGYGRSIRIVGEDNKLTAEMQLSRVRSDIVSRHGQALLNK
 PHGLKLEKMEPVTDLNPKLWYIAYQLREKKRYHYGVFSTSYIEEHNSRIEASRIKTNKWIPVCPAISK
 QSSDGKPSLAKIPMLNIGEIKFTKLQIAVDDHAMIRKAPFSKMVFFDGPPIQSGGIDIGKLMKNQNILNL
 RLDNIQSITLLDLCRIFSCRGSKVDQDAFEFLSDEPLDEDVIDELDSSPALVVSYTKKSTKSNSFKNVIV
 RALIRECDIFEDIMDITDDGFTSDSNLEVLENLTWILNMLATNQWSTELLACIHMCLYRNEMDHIYHNFQ
 VPEIFVDNPISLNVKWDEVIMFLNILRDRDYKFEPWVSILNHSITKAIEYAYKKMEERKQKSTGINKFL
 KGKKMGGRSKFDFQ

FIG. 5 (3/4)

SEQ ID NO:16 ("L" 3'UTR)

TAGCTTGATCTTAAATAATACATAATCTTTGCCCCAAATCTGTATTATATAAATAATTCTAAAGTAGTTT
CATGTAATTAGGGGCACACTACT

SEQ ID NO:6 (Ribozyme/T7 Terminator)

GGGTCGGCATGGCATCTCCACCTCCTCGCGGTCCGACCTGGGCATCCGAAGGAGGACGTCGTCCACTCGG
ATGGCTAAGGGAGAGCTCGGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACC
GCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

FIG. 5 (4/4)

SEQ ID NO:17 (HE649912 nucleotide)

CTAATTACAATCACTATGGAGACATACAAGATTAACATTTTTAGAGATAGGATCAACCAGTGTCGAAGTGCTGA
AGAAGCCAAAGACATTGTTGCTGATCTTCTCATGGCTAGACATGACTACTTTGGTAGAGAGGTATGTTATTACC
TGGATATCGAATTCCGGCAGGATGTTCCAGCTTACGACATACTTCTTGAATTTCTGCCAGCTGGCACTGCTTTC
AACATTGCAATTGTACACCAGACAATTTTATCATTACAAATGGCAAGCTTTATATCATTGACTATAAAGTATC
AACTGATCATGCATATGGTCAAAAACTTATGAAAAGTACACCCAGATCTTTGGAGACGCATTGTCAGAATTGC
CGTTTGATTTTGAAGTTGTGATCATCCGTGCTGACCCTCTGCGAGATACTATCCATGTTAATCAAATCAATTC
TTGGAAATATTTGGGCCGCTCAACATAAACCTTGATTTTACTTGGTCTTTAATTTGCGATCCCTGATATATGA
GAAATATAAGGATGACGACAGATTCCAGAAAATTGTGAATCAAGGTGAATTTACGATGACTGGACCCTGGATTG
ATGAGGATACCCCGGAGCTCTATTACACCCCTGTCTTTTTGGAATTCATGATTCTTTAGATGAGATGGCTAAA
CTGACATTCCATGAGTCTATGACATTTGATGCAACTCGCGGTGAGAAAATGGAATCAAAATCTACAAAAGGTTAT
AAATAGATATGGCAATGATTATAACATTTTTGTGAAAGAGGCCGCTGCAGGAATCTTTAGATGTGAAGGGAAC
ACCCAAAACCAATCATGATGAAATCACAATCGGTTGGAATCAAATGGTTCAAAGAGTGAGTACTGAGAGAAAC
CTGACTCAAGATGTCAGCAAGCAAAAACCATCTATTCATTTTCATATGGGGTCAACCTGACGAAACATCAAATGC
GACAACACCAAACTAATCAAGATTGCAAAAAGCACTCCAAAATATTTCTGGCGAGTCTACATATATAAGCGCAT
TCAGAGCATTGGGTATGCTTATGGACTTTTCTGAGAACACAGCTTTATATGAAGCACACACTAGCAAACTAAAA
AGTATGGCAAGACAGACATCGAAAAGAATTGATACTAACTGGAACCAATCAAAATAGGCACGCGACAATTTA
TTGGGAACAGCAGTTTAACTGGATACTGAAATAATGAATACAAAAGACAAATCACATTTGCTAAAAGATTTTC
TTGGCATAGGGGTCACGTGCAATTTTCAAAAAGACCATTGACGATTTGGATACTGACAAACCTACTATATTA
GATTTCAACAAAAGGAAGTCATTGATTTTTCGAAATTCAGTATGAAAATGTAAAGAAAATACTATCCGGAGA
TAATAATCTAGAGCGTATAGGATGTTATTTAGAAGAATATGGTGCAAATATTGCATCATGTTCAAAGGATACAT
GGGATCAGATTAACCAGATAGGGAAGTCAAATTACTGGGCTTGATTTAAAGATTTTTTCACTCTTGATGAAAAT
ATGTTGGCAGTTTCTCAATATAATAGGCACAATACTTTTCGTGTAGTGTGTTGTGCAACAATAATCTGTTTGG
GTTTGTAATGCCTTCTTCTGATATTAAAGCAAAGCGATCCACACTTGTTTACTTCTTAGCTGTGTTGCATTCTA
CTCCTCAGAATGTGATGCACCACGGTGCATTGCATGCGACATTTAAACTGGTTCAAATACCTTAGTATCTCT
AAAGGAATGCGTTTAGATAAAGAACGATGTCAACGCATAGTTAGTTTACCGGGACTTTTTATGTTGACTACATT
GATGTTTGCAGGAGACAATCCGACACTCAATTTGACTGATGTCATGAATTTTACATTCCACACTCCCTGTCTA
TAACCAAAGCTATGCTGTCATTGACAGAACCATCAAGATATATGATAATGAATTCATTAGCCATATCCAGTCAT
GTTAGAGATTATATAGCAGAAAAATTTGGCCCTTATACAAAGACCAGCTTCTCTGTAGTAATGGCAAACCTGAT
TAAAAGGGGATGTTATATGGCATATAATCAAAGAGATAAAGTAGACATGAGGAATATCTGCCTAACAGATTATG
AAATAACTCAAAAAGGTGTGAGAGATAACAGAGACCTATCATCAATCTGGTTTGAAGGCTATGTATCACTAAAA
GAATATATTAACCAAATATATCTACCATTTTACTTCAATTCAAAAGGTTTGCATGAAAAGCATCATGTTATGAT
AGATCTGGCTAAGACAATCTTAGATATAGAAAGGGACCAGAGATTAATATCCCAGGAATATGGTCTACAACAC
CTAGAAAACAACTGCAAATTTAAATATAACTATCTATGCAGTTGCAAAAAATCTAATAATGGACACTGCTAGA
CATAATTATATTAGATCACGGATAGAAAACACAAACAACTTAAATAGATCGATATGCACTATTTCTACATTCAC
CAGCTCTAAATCATGTATTAAAGTAGGCGACTTTGAGAAAGAAAAAGCTCAGCAACAAAAAAGGCTGCAGATT
GCATGTCAAAAAGAGATAAAGAAGTATACAATTGCAACCCAGAATTTGTTGATGAAGAGTTACTAAATGCAACT
ATAAGACATTACGCTATGAAGACTTAAAAAAGCAATCCCGAATTATATTGACATTATGTCAACTAAAGTATT
TGATTCTCTGTACCAGAAAATAAAAAGGAAGGAGATAGATGATAAAACCACTGTGTATCATATACTCTCTGCTA
TGAAGAATCACACAGATTTTAAAGTTTACATTCTTTAACAAGGCCAAAAACAGCAAAGGATAGGGAAATATTC
GTAGGCGAATTTGAGGCAAAAATGTGCTTGATTTTAGTGAGAGGATATCTAAAGAACGCTGTAAGTTGAATCC
AGATGAGATGATTAGTGAACCAGGCGATTCTAAATTGAAAAAATTAGAAGAGCTTGCAAGTCTGAAATACGAT
TCACAGCAGCAACTATGAAACAGATCAAAGAACGCTATTTAGCAGAAATGGGAGAAGCAAGCCATATGATCGCA
TATAAACCACATTCTGTTAAGATTGAAATCAATGCAGACATGTCAAAATGGAGTGCCCAAGATGTTTTATTCAA
ATATTTCTGGTTGTTTGCATTAGATCCCGCACTTTATCTGCAAGAAAAAGAAAGGATATTGTACTTCCTATGCA
ATTATATGCAAAAAAAGCTAATTCTGCCTGATGAAATGCTCTGTAGCATCCTTGACCAACGTATCAAACATGAG
GATGATATAATATATGAAATGACCAATGGCTTATCGCAAAATTTGGGTCAATATTAAACGGAACCTGGCTGCAGGG
GAATCTCAATTACACAAGTAGCTACCTACATTCATGTTCTATGAATGTTTATAAGGATATTCTAAAGAGAGCAG
CCACTTTACTAGAAGGGGAAGTTTTAGTCAATTCATGTTTCATTCTGATGACAATCACACTTCAATAGTGATG
ATCCAAGATAAATTAGATGATGATATTGTTATTGAATTTTCTGCAAACTATTTGAAAAAATATGTCTAACTTT

FIG. 6 (1/2)

TGGAAATCAAGCAAATATGAAGAAGACATATATAACAAATTTTCATAAAGGAGTTCGTTTCACTTTTTAATATTT
ATGGTGAGCCATTTTCTGTTTATGGTCGCTTTATTTTGACATCTGTTGGCGATTGTGCTTTTCTTGGACCATAT
GAGGATGTTGCCAGTAGGTTGTCTGCAACGCAGACAGCAATTAAGCATGGAGCACCTCCATCACTTGCATGGAC
TGCTATTGCATTAACCTCAGTGGATAACACATAGCACATATAACATGCTTCCAGGTCAAATCAATGATCCTACTT
CATCTTTACCTAGTCATGATAGATTTGAGCTGCCTATAGAATTGTGTGGCTTAATAAATTCAGAATTACCCACT
ATAGCTATAGCAGGTTTGGGAAGCAGATAATCTAAGTTATTTAGTTAGGTTATCAAAAAGAATGTCCCCTATACA
TCTTTGCCGTGAACCAATCCAGCATCAATATGAGAATATACATACATGGGATATAAGTAACTGACACAATGTG
ATATTTTCAGACTTAAGCTTTTAAGATACATGACGTTAGACTCAACTATGTCATCTGATGATGGAATGGGCGAA
ACTAGTGAAATGAGATCTAGGTCTCTTCTGACACCAAGAAAATTCACTACTGCAAGTTCGTTATCTAGATTGCA
TTCATATGCTGATTATCAAAAAACAATACAAGACCAACAGAAAATTGAAGAATTATTTGAATATTTTATAGCCA
ACCCTCAACTATTGGTTACAAAAGGTGAGACTTGTGAAGAGTTCTGTATGTCTGTATTGTTTCAGATACAACAGT
CGTAAATTTAAAGAATCATTGTCTATTCAAAACCCAGCTCAGCTCTTCATAGAACAAGTATTGTTTGCAAATAA
ACCAATGATAGACTATACAAGTATTCATGATAGGTTGTTTGGTATACAAGATGACCCAAATATAAATGATGCTA
CATGTATTATTGGCAAGAAGACTTTTGTGAAACATATCAGCAATAAAAAATTGATGTAGAAAAATTTACACTT
GATGTAGAGGATATAAAGACGATATATAGCTTCTGTATAATGAACGACCCTATATTAGTTGCTTGTGCAAACAA
CTTGTTAATTTCAATACAGGGAGTGGAGATGCAACGATTGGGTATGACATGCTGTTATATGCCGGAGATTAAGA
GCCTTAAAGTAATTTATCATAGTCCTGCTCTCGTATTACGTGCTTATGTAACAGATAACTATGAGCAAAAAGGG
ATGGAGCCAGATGAAATGCGGAGAGATATATATCATTTAGAAGAATTTATAGAGAAGACAAAAATTGAGGACAAA
TATGCAAGGGGAGAATTGCAAATAATGAAATTAAGTTAATGAAGCGAGATTTGAAATTTGAAGTGCAGGAATTGA
CTAAATTTCTATCAGATCTGTTATGAATATGTGAAATCAACAGAACACAAAATTAATAATTTTCATCCTTCCAAA
AAGGCTTACACTCCCATTGATTTCTGCTCATTAGTAACAGGTAATCTGATATCAGACAACAAATGGATGGTTGT
TCACTATTTAAACAAATAACTGTCCCAGCAAAGAAGGCACAAATAGCCACATCTATAGATCTGGAAATACAAA
TAGCCTACGAATGTTTCAGGCTAATTGCACATTTTGTGATATGTTTCTTAAATGATGACTCCAAAAAAGCTTAT
ATTAATGCAATTATTAACACATATACATACAAGGATGTTCAAGTATCCAGTCTCTACAAGAAAATCAAAAATTC
GAGACTACGTTCAAAAATTATACCATTATTATATCACCTGGGCGATTGCAACAAATAGACGTTGACAGATTTG
ATGCAGAAAAAGCAGAAGAGCAGATCACATGGAATAACTGGCAAACATCTCGAGAATTTACTACTGGTCCAATT
GATCTATCAATCAAAGGTTATGGACGGTCAATAAGGATCGTAGGTGAGGACAACAAGCTTACAGCTGCAGAAAT
GCAATTGTCAAGAGTGAGAAGTGATATAGTATCAAGGCATGGACAGGCTTTATTGAACAAACCTCATGGGCTAA
AATTAGAGAAAAATGGAACCAAGTGAAGTCTAAATCCTAAATTATGGTATATTGCATACCAATTGCGTGAGAAA
AAGCGGTATCACTATGGGGTCTTTAGTACATCTTATATAGAAGAGCATAACTCAAGGATAGAAGCATCTCGGAT
ACGTAAGACTAATAAATGGATACCAGTTTGCCCTATTGCTATATCAAAAACAATCATCTGATGGAAAGCCTAGTC
TTGCAAAAATCCCTATGTTAAATATTGGGGAGATTAAATTTACAAAACCTACAGATTGCAGTAGATGATCATGCA
ATGATTAGGAAAGCCCCATTTAGTAAGATGGTGTCTTTGATGGCCCCACCCATACAGAGCGGTGGCATTGACAT
TGGAAGCTTATGAAGAACCAAAATATTCTCAATTTGAGGTTAGATAATATACAGAGTATAACATTATTAGATT
TGTGCCGCATATTTTCATGCCGAGGGTCTAAAGTGGATCAAGATGCATTTGAATTCTTATCTGATGAACCTTTG
GATGAAGATGTTATTGATGAATTAGATAGCTCACCTGCATTAGTGGTATCTTACACAAAGAAATCAACCAAATC
CAATAGTTTCAAAAATGTTATAGTTAGAGCATTGATAAGAGAATGTGATATATTTGAAGATATAATGGACATAA
CAGACGATGGATTCACATCTGATAGCAATCTAGAGGTGTTAGAAAACCTAACATGGATTTTAAATATGCTCGCA
ACAAATCAGTGGTCTACAGAACTGTTAGCATGCATACACATGTGTTTATATCGCAATGAGATGGATCATATCTA
TCACAATTTTCAAGTTCAGAAATATTTGTGCGACAATCCAATCTCATTAATGTAAAGTGGGATGAAGTAATTA
TGTTCTTAAACATACTGCGAGACAGAGATTACAAATTTGAGCCATGGGTGTCTATACTGAATCATTCCTTAACT
AAAGCTATAGAGTATGCTTACAAAAGATGGAAGAGGAGAGGAAGCAGAAATCAACAGGCATCAACAAATTCCTT
AAAGGGTAAAAAATGGGTGGCAGATCAAAGTTTGATTTCCAGTAGCTTGATCTTAAATAATACATAATCTTTG
CCCCAAATCTGTATTATATAAATAATTCTAAAGTAGTTTCATGTAATTAGGGGCAC

FIG. 6 (2/2)

SEQ ID NO:18 (HE649912 amino acid)

METYKINIFRDRINQCRSAEEAKDIVADLLMARHDYFGREVCYYLDIEFRQDVPAYDILLEFLPAGTAFNIRNC
 TPDNFIHNGKLYIIDYKVSTDHAYGQKTYEKYTQIFGDALSELPDFEVVIRADPLRDTIHVNSNQFLEIFG
 PLNINLDFTWFFNLRLSLIYEKYKDDDRFLEIVNQGEFTMTGPWIDEDTPELYSHPVFLEFYDSLDEMAKLTFFE
 SMTFDATRGEKWNQNLQKVINRYGNDYNI FVKEAAAGIFRCEGNYPKPNHDEITIGWNQMVQRVSTERNLTQDV
 SKQKPSIHFIWGQPDETS NATTPKLIKIAKALQNISGESTYISAFRALGMLMDFSENTALYEAHTSKLKSMARQ
 TSKRIDTKLEPIKIGTATIYWEQQFKLDTEIMNTKDKSHLLKDFLGIGGHVQFSKKTIDDLDTDKPTILDFNKK
 EVIDFCKFQYENVKILSGDNNLERIGCYLEEYGANIASCSKDTWDQINQIGKSNYWACIKDFSVLKMNMLAVS
 QYNRHNTFRVVCANNLFGFVMPSSDIKAKRSTLVYFLAVLHSTPQNVMHGALHATFKTGSKYLSISKGMRL
 DKERCQRIVSSPGLFMLTTLMFAGDNPTLNLTDMNFTFHTSL SITKAMLSLTEPSRYMIMNSLAISSHVRDYI
 AEKFGPYTKTSFSVVMANLIKRGCYMAYNQDKVDMRNICLT DYEITQKGVDRNDRDLSSIWFEQYVSLKEYINQ
 IYLPFYFNSKGLHEKHHVMIDLAKTILDIERDQRLNIPGIWSTTPRKQTANLNITIYAVAKNLMIDTARHNYIR
 SRIENTNNLNRSICTISTFTSSKSCIKVGD FEKEKSSATKKAADCMSKEIKKYTIANPEFVDEELNATIRHSR
 YEDLKKAI PN YIDIMSTKVFD SLYQKIKRKEIDDKPTVYHILSAMKNHTDFKFTFFNKGQKTAKDREIFVGEFE
 AKMCLYLVERISKERCKLNPDEMISEPGDSKLKLEELAESEIRFTAATMKQIKERYLAEMGEASHMIAYKPHS
 VKIEINADMSKWSAQDVLFKYFWLFALDPALYLQEKERILYFLCNYMQKKLILPDEMLCSILDQRIKHEDDIY
 EMTNGLSQNWVNIKRNLWLGNLNYTSSYLHSCSMNVYKDILKRAATLLEGEVLVNSMVHSDDNHTSIVMIQDKL
 DDDIVIEFSAKLFEKICLTFGNQANMKKTYITNFIKEFVSLFNIYGEPPFSVYGRFILTSVGDCAFLGPYEDVAS
 RLSATQTAIKHGAPPSLAWTAIALTQWITHTSYNMLPGQINDPTSSLPSHDFELPIELCGLINSELPTIAIAG
 LEADNLSYLVRLSKRMSPHLCREPIQH QYENIHTWDISKLTQCDIFRLKLLRYMTLDSTMSSDDGMGETSEMR
 SRSLTTPRKFTTASSLSRLHSYADYQKTIQDQQKIEELFEYFIANPQLLVTKGETCEEFCMSVLFYNSRKFKKE
 SLSIQNPAQLFIEQVLFANKPMIDYTSIHDRLFGIQDDPNINDATCIIGKKT FVETYQQIKIDVEKFTLDVEDI
 KTIYSFCIMNDPILVACANNLLISIQGVEMQRLGMTCCYMP EIKSLKVIYHSPALVLRAYVTDNYEQKGMEPDE
 MRRDIYHLEEFIEKTKLRNTMQGRIANNEIKLMKRD LKFEVQELTKFYQICYEYVKSTEHKIKIFILPKKAYTP
 IDFCSLVTGNLISDNKWMVHYLQKITVPAKKAQIATSIDLEIQIAYECFRLIAHFADMFLNDDSKKAYINAI I
 NTTYTYKDVQVSSLYKKIKNSRLRSKIIPLLYHLGDLQQIDVDRFDAEKAEEQITWNNWQTSREFTTGPIDLSIK
 GYGRSIRIVGEDNKLTA AEMQLSRVRSDIVSRHGQALLNKPHGLKLEKMEPVTDLNPKLWYIAYQLREKKRYHY
 GVFTSYIEEHNSRIEASRIRKTNKWIPVCPIAISKQSSDGKPSLAKIPMLNIGEIKFTKLQIAVDDHAMIRKA
 PFSKMVFFDGPPIQSGGIDIGKLMKNQNILNLRLDNIQSITLLDLCRIFSCRGSKVDQDAFEFLSDEPLDEDVI
 DELDSSPALVVSYTKKSTKSNSFKNVIVRALIRECDIFEDIMDITDDGFTSDSNLEVLENLTWILNMLATNQWS
 TELLACIHMCLYRNEMDHIYHNFQVPEIFVDNPI SLNVKWDEVIMFLNILRDRDYKFEPWVSILNHSLTKAIEY
 AYKKMEEERKQKSTGINKFLKGKKMGGRSKFDFQ

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/023243

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/12 C07K14/00 C12N7/00 C12N15/86
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K C12N

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Date of the actual completion of the international search

27 August 2014

Date of mailing of the international search report

05/09/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Cilensek, Zoran

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/023243

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INTERNATIONAL SEARCH REPORT

International application No

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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International application No

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