

REPLICATION-DEFECTIVE FLAVIVIRUS VACCINES
AND VACCINE VECTORS

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Field of the Invention

This invention relates to replication-defective flavivirus vaccines and vaccine vectors, and corresponding compositions and methods.

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Background of the Invention

Flaviviruses are distributed worldwide and represent a global public health problem. Flaviviruses also have a significant impact as veterinary pathogens. Flavivirus pathogens include yellow fever (YF), dengue types 1-4 (DEN1-4), Japanese encephalitis (JE), West Nile (WN), tick-borne encephalitis (TBE), and other viruses from the TBE serocomplex, such as Kyasanur Forest disease (KFD) and Omsk hemorrhagic fever (OHF) viruses. Vaccines against YF [live attenuated vaccine (LAV) strain 17D], JE [inactivated vaccines (INV) and LAV], and TBE (INV) are available. No licensed human vaccines are currently available against DEN and WN. Veterinary vaccines have been in use including, for example, vaccines against WN in horses (INV, recombinant and live chimeric vaccines), JE (INV and LAV) to prevent encephalitis in horses and stillbirth in pigs in Asia, louping ill flavivirus (INV) to prevent neurologic disease in sheep in the UK, and TBE (INV) used in farm animals in Czech Republic (INV) (Monath and Heinz, Flaviviruses, in Fields et al. Eds., Fields Virology, 3rd Edition, Philadelphia, New York, Lippincott-Raven Publishers, 1996, pp. 961-1034).

Tick-borne encephalitis (TBE) is the most important tick-borne viral disease of humans. It is endemic in parts of Europe and Northern Asia, causing more than 10,000 hospitalizations annually, with a case-fatality rate 0.5–1.5% in Europe and 6–40% in Siberia and the Far East. A significant proportion of patients suffer from long-lasting neuropsychiatric sequelae. Inactivated vaccines produced in chick embryo cell cultures have proven effective in preventing the disease. For example, an 86% vaccination coverage of the Austrian population (the highest among European countries) has resulted

in an approximately 90% reduction of hospitalized cases (Heinz and Kunz, Arch. Virol. Suppl. 18:201-205, 2004). The inactivated vaccines are expensive and require three inoculations for primary immunization. Periodic boosters (every 2-5 years) are required to maintain immunity. Therefore, a less costly TBE vaccine, which is effective after one-
5 two doses and provides durable, such as life-long immunity (similar to that achieved by YF 17D immunization) is needed, and indeed has been identified by the WHO as a major priority. Development of TBE LAV candidates in the past several decades by means of empirical or rational attenuation of TBE virus parent *per se* or chimerization of TBE or Langat (LGT, a naturally attenuated flavivirus that is closely related (serologically) to
10 TBE) viruses with dengue 4 virus has faced difficulties due to problems with residual virulence of candidates and/or low immunogenicity/overattenuation (Wright et al., Vaccine 26:882-890, 2008; Maximova et al., J. Virol. 82:5255-5268, 2008; Rummyantsev et al., Vaccine 24:133-143, 2006; Kofler et al., Arch. Virol. Suppl. 18:191-200, 2004; and references therein).

15 Flaviviruses are small, enveloped, plus-strand RNA viruses transmitted primarily by arthropod vectors (mosquitoes or ticks) to natural hosts, which are primarily vertebrate animals, such as various mammals, including humans, and birds. The flavivirus genomic RNA molecule is about 11,000 nucleotides (nt) in length and encompasses a long open reading frame (ORF) flanked by 5' and 3' untranslated terminal regions (UTRs) of about
20 120 and 500 nucleotides in length, respectively. The ORF encodes a polyprotein precursor that is cleaved co- and post-translationally to generate individual viral proteins. The proteins are encoded in the order: C-prM/M-E-NS1-NS2A/2B-NS3-NS4A/4B-NS5, where C (core/capsid), prM/M (pre-membrane/membrane), and E (envelope) are the structural proteins, i.e., the components of viral particles, and the NS proteins are non-
25 structural proteins, which are involved in intracellular virus replication. Flavivirus replication occurs in the cytoplasm. Upon infection of cells and translation of genomic RNA, processing of the polyprotein starts with translocation of the prM portion of the polyprotein into the lumen of endoplasmic reticulum (ER) of infected cells, followed by translocation of E and NS1 portions, as directed by the hydrophobic signals for the prM,
30 E, and NS1 proteins. Amino-termini of prM, E, and NS1 proteins are generated by

cleavage with cellular signalase, which is located on the luminal side of the ER membrane, and the resulting individual proteins remain carboxy-terminally anchored in the membrane. Most of the remaining cleavages, in the nonstructural region, are carried out by the viral NS2B/NS3 serine protease. The viral protease is also responsible for
5 generating the C-terminus of the mature C protein found in progeny virions. Newly synthesized genomic RNA molecules and the C protein form a dense spherical nucleocapsid, which becomes surrounded by cellular membrane in which the E and prM proteins are embedded. The mature M protein is produced by cleavage of prM shortly prior to virus release by cellular furin or a similar protease. E, the major protein of the
10 envelope, is the principal target for neutralizing antibodies, the main correlate of immunity against flavivirus infection. Virus-specific cytotoxic T-lymphocyte (CTL) response is the other key attribute of immunity. Multiple CD8+ and CD4+ CTL epitopes have been characterized in various flavivirus structural and non-structural proteins. In addition, innate immune responses contribute to both virus clearance and regulating the
15 development of adaptive immune responses and immunologic memory.

In addition to the inactivated (INV) and live-attenuated (LAV) vaccines against flaviviruses discussed above, other vaccine platforms have been developed. One example is based on chimeric flaviviruses that include yellow fever virus capsid and non-structural sequences and prM-E proteins from other flaviviruses, to which immunity is sought. This
20 technology has been used to develop vaccine candidates against dengue (DEN), Japanese encephalitis (JE), West Nile (WN), and St. Louis encephalitis (SLE) viruses (see, e.g., U.S. Patent Nos. 6,962,708 and 6,696,281). Yellow fever virus-based chimeric flaviviruses have yielded highly promising results in clinical trials.

Another flavivirus vaccine platform is based on the use of pseudoinfectious
25 virus (PIV) technology (Mason et al., Virology 351:432-443, 2006; Shustov et al., J. Virol. 21:11737-11748, 2007; Widman et al., Adv. Virus. Res. 72:77-126, 2008; Suzuki et al., J. Virol. 82:6942-6951, 2008; Suzuki et al., J. Virol. 83:1870-1880, 2009; Ishikawa et al., Vaccine 26:2772-2781, 2008; Widman et al., Vaccine 26:2762-2771, 2008). PIVs are replication-defective viruses attenuated by a deletion(s). Unlike live flavivirus
30 vaccines, they undergo a single round replication *in vivo* (or optionally limited rounds, for

two-component constructs; see below), which may provide benefits with respect to safety. PIVs also do not induce viremia and systemic infection. Further, unlike inactivated vaccines, PIVs mimic whole virus infection, which can result in increased efficacy due to the induction of robust B- and T-cell responses, higher durability of immunity, and
5 decreased dose requirements. Similar to whole viruses, PIV vaccines target antigen-presenting cells, such as dendritic cells, stimulate toll-like receptors (TLRs), and induce balanced Th1/Th2 immunity. In addition, PIV constructs have been shown to grow to high titers in substrate cells, with little or no cytopathic effect (CPE), allowing for high-yield manufacture, optionally employing multiple harvests and/or expansion of infected
10 substrate cells.

The principles of the PIV technology are illustrated in Figs. 1 and 2. There are two variations of the technology. In the first variation, a single-component pseudoinfectious virus (s-PIV) is constructed with a large deletion in the capsid protein (C), rendering mutant virus unable to form infectious viral particles in normal cells (Fig.
15 1). The deletion does not remove the first ~20 codons of the C protein, which contain an RNA cyclization sequence, and a similar number of codons at the end of C, which encode a viral protease cleavage site and the signal peptide for prM. The s-PIV can be propagated, e.g., during manufacture, in substrate (helper) cell cultures in which the C protein is supplied in trans, e.g., in stably transfected cells producing the C protein (or a
20 larger helper cassette including C protein), or in cells containing an alphavirus replicon [e.g., a Venezuelan equine encephalitis virus (VEE) replicon] expressing the C protein or another intracellular expression vector expressing the C protein. Following inoculation in vivo, e.g., after immunization, the PIV undergoes a single round of replication in infected cells in the absence of trans-complementation of the deletion, without spread to
25 surrounding cells. The infected cells produce empty virus-like particles (VLPs), which are the product of the prM-E genes in the PIV, resulting in the induction of neutralizing antibody response. A T-cell response should also be induced via MHC I presentation of viral epitopes. This approach has been applied to YF 17D virus and WN viruses and WN/JE and WN/DEN2 chimeric viruses (Mason et al., *Virology* 351:432-443, 2006;

Suzuki et al., J. Virol. 83:1870-1880, 2009; Ishikawa et al., Vaccine 26:2772-2781, 2008; Widman et al., Vaccine 26:2762-2771, 2008; WO 2007/098267; WO 2008/137163).

In the second variation, a two-component PIV (d-PIV) is constructed (Fig. 2). Substrate cells are transfected with two defective viral RNAs, one with a deletion in the C gene and another lacking the prM-E envelope protein genes. The two defective genomes complement each other, resulting in accumulation of two types of PIVs in the cell culture medium (Shustov et al., J. Virol. 21:11737-11748, 2007; Suzuki et al., J. Virol. 82:6942-6951, 2008). Optionally, the two PIVs can be manufactured separately in appropriate helper cell lines and then mixed in a two-component formulation. The latter may offer an advantage of adjusting relative concentrations of the two components, increasing immunogenicity and efficacy. This type of PIV vaccine should be able to undergo a limited spread in vivo due to coinfection of some cells at the site of inoculation with both components. The spread is expected to be self-limiting as there are more cells in tissues than viral particles produced by initially coinfecting cells. In addition, a relatively high MOI is necessary for efficient co-infection, and cells outside of the inoculation site are not expected to be efficiently coinfecting (e.g., in draining lymph nodes). Cells infected with the ΔC PIV alone produce the highly immunogenic VLPs. Coinfecting cells produce the two types of packaged defective viral particles, which also stimulate neutralizing antibodies. The limited infection is expected to result in a stronger neutralizing antibody response and T-cell response compared to s-PIVs. To decrease chances of recombination during manufacture or in vivo, including with circulating flaviviruses, viral sequences can be modified in both s-PIVs and d-PIVs using, e.g., synonymous codon replacements, to reduce nucleotide sequence homologies, and mutating the complementary cyclization 5' and 3' elements.

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Summary of the Invention

The invention provides replication-deficient or defective pseudoinfectious flaviviruses including a flavivirus genome that includes (i) one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1

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(NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), and (ii) sequences encoding one or more heterologous pathogen, cancer, or allergy-related immunogens. For example, the deletion/mutation can be within capsid (C) sequences; pre-membrane (prM) and/or envelope (E) sequences; capsid (C), pre-membrane (prM),
5 and envelope (E) sequences; or non-structural protein 1 (NS1) sequences.

The heterologous immunogen can be, for example, from a pathogen selected from the group consisting of a rabies virus (e.g., a rabies virus G protein epitope), *Borrelia burgdorferi* (e.g., OspA immunogen or an immunogenic fragment thereof), a tick (e.g., a tick saliva protein selected from the group consisting of 64TRP, Isac, and Salp20, or an
10 immunogenic fragment thereof), an influenza virus (e.g., an influenza virus M2, hemagglutinin (HA), or neuraminidase (NA) epitope, or an immunogenic fragment thereof), a human immunodeficiency virus (e.g., a codon-optimized HIV gag, tat/nef, or gp120 protein, or an immunogenic fragment thereof), a simian immunodeficiency virus, a human papilloma virus (e.g., an HPV16 or HPV18 capsid protein L1 or L2, or an
15 immunogenic fragment thereof), a respiratory syncytial virus (e.g., a respiratory syncytial virus F or G glycoprotein), malaria parasite, and *Mycobacterium tuberculosis* (also see below).

The replication-deficient pseudoinfectious flaviviruses can include sequences encoding a pre-membrane (prM) and/or envelope (E) protein. Further, the replication-
20 deficient pseudoinfectious flavivirus genomes can be selected from those of yellow fever virus, West Nile virus, tick-borne encephalitis virus, Langat virus, Japanese encephalitis virus, dengue virus, and St. Louis encephalitis virus, attenuated strains thereof, and chimeras thereof (also see below). In various examples, the chimeras include pre-membrane (prM) and envelope (E) sequences of a first flavivirus (e.g., a tick-borne
25 encephalitis virus or a Langat virus), and capsid (C) and non-structural sequences of a second, different flavivirus (e.g., a yellow fever, a West Nile, or Langat virus).

The replication-deficient pseudoinfectious flavivirus genomes can be packaged in particles including pre-membrane (prM) and envelope (E) sequences from a flavivirus that is the same or different from that of the genomes. Further, the sequences encoding

the heterologous immunogens can be inserted in the place of, or in combination with, the deletion(s) or mutation(s) of the one or more proteins.

The sequences encoding the heterologous immunogens can be inserted in the flavivirus genomes within sequences encoding the envelope (E) protein, within sequences
5 encoding the non-structural 1 (NS1) protein, within sequences encoding the pre-membrane (prM) protein, intergenically between sequences encoding the envelope (E) protein and non-structural protein 1 (NS1), intergenically between non-structural protein 2B (NS2B) and non-structural protein 3 (NS3), and/or as a bicistronic insertion in the 3' untranslated region of the flavivirus genome.

10 The invention also includes compositions including a first replication-deficient pseudoinfectious flavivirus, as described above, and a second (or further), different replication-deficient pseudoinfectious flavivirus including a genome that includes one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E),
15 non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5). In these compositions, the one or more proteins encoded by the sequences in which the deletion(s) or mutation(s) occur in the second, different replication-deficient pseudoinfectious flavivirus are different from the one or more proteins encoded by the sequences in which the deletion(s) occur in the first replication-deficient pseudoinfectious
20 flavivirus.

The invention further includes methods of inducing immune responses to an immunogen in a subject, which involves administering to the subject one or more replication-deficient pseudoinfectious flavivirus and/or composition as described herein to the subject. In various examples, the subject is at risk of but does not have an infection
25 by the pathogen or a disease or condition associated with the cancer or allergy-related immunogen. In other examples, the subject has an infection by the pathogen or a disease or condition associated with the cancer or allergy-related immunogen. The invention thus includes prophylactic and therapeutic methods. In these methods, the immunogen can be from, for example, a pathogen selected from the group consisting of a rabies virus,
30 *Borrelia burgdorferi*, a tick, an influenza virus, a human immunodeficiency virus, a

simian immunodeficiency virus, a human papilloma virus, a respiratory syncytial virus, malaria parasite, and *Mycobacterium tuberculosis* (also see below). Further, the methods can be for inducing an immune response against a protein encoded by the flavivirus genome, in addition to the source of the immunogen. In various examples, the subject is
5 at risk of but does not have an infection by the flavivirus corresponding to the genome of the pseudoinfectious flavivirus, which includes sequences encoding a flavivirus pre-membrane and/or envelope protein. In other examples, the subject has an infection by the flavivirus corresponding to the genome of the pseudoinfectious flavivirus, which includes sequences encoding a flavivirus pre-membrane and/or envelope protein.

10 The invention also includes live, attenuated chimeric flaviviruses including a yellow fever virus in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis virus or a Langat virus, and the signal sequence between the capsid and pre-membrane proteins of the chimeric flavivirus includes a hybrid of yellow fever virus and
15 tick-borne encephalitis or Langat virus capsid/pre-membrane signal sequences, or a variant thereof. In various examples, the capsid/pre-membrane signal sequence of the chimeric flavivirus includes yellow fever virus sequences in the amino terminal region and tick-borne encephalitis or Langat virus sequences in the carboxy terminal region (see below).

20 Further, the invention includes live, attenuated chimeric flaviviruses including a West Nile virus in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis or a Langat virus, and the signal sequence between the capsid and pre-membrane proteins of the chimeric flavivirus includes a tick-borne encephalitis or a
25 Langat virus capsid/pre-membrane signal sequence, or a variant thereof.

The invention also includes pharmaceutical compositions including one or more pseudoinfectious flavivirus, composition, or live, attenuated flavivirus as described herein, and a pharmaceutically acceptable carrier or diluent. Further, the compositions can include an adjuvant.

Also included in the invention are replication-deficient pseudoinfectious flaviviruses including a flavivirus genome including one or more deletion(s) or mutation(s) in nucleotide sequences encoding non-structural protein 1 (NS1), non-structural protein 3 (NS3), or non-structural protein 5 (NS5).

5 Further, the invention includes nucleic acid molecules corresponding to the genome of a pseudoinfectious flavivirus, or the genome the live, attenuated flavivirus, as described herein, and complements thereof.

The invention also provides methods of making replication-deficient pseudoinfectious flaviviruses as described herein, involving introducing one or more
10 nucleic acid molecules, as described above, into a cell that expresses the protein(s) corresponding to any sequences deleted from the flavivirus genome of the replication-deficient pseudoinfectious flaviviruses. In these methods, the protein can be expressed in the cell from the genome of a second (or further), different, replication-deficient pseudoinfectious flavivirus. In other examples, the protein is expressed from a replicon
15 (e.g., an alphavirus replicon, such as a Venezuelan Equine Encephalitis virus replicon; see below).

The invention also includes compositions containing two or more replication-deficient pseudoinfectious flaviviruses, in which two of the replication-deficient pseudoinfectious flaviviruses are selected from the groups consisting of: (a) a replication-
20 deficient pseudoinfectious flavivirus including a genome containing Japanese encephalitis virus sequences, and a replication-deficient pseudoinfectious flavivirus including a genome containing dengue virus sequences; (b) a replication-deficient pseudoinfectious flavivirus including a genome containing yellow fever virus sequences, and a replication-deficient pseudoinfectious flavivirus including a genome containing dengue virus
25 sequences; and (c) a replication-deficient pseudoinfectious flavivirus including a genome containing tick-borne encephalitis or Langat virus sequences and an inserted sequence encoding a *Borrelia burgdorferi* immunogen, and a replication-deficient pseudoinfectious flavivirus including a genome containing tick-borne encephalitis or Langat virus
30 sequences and an inserted sequence encoding a tick saliva protein immunogen, or a replication-deficient pseudoinfectious flavivirus including a genome containing

tick-borne encephalitis or Langat virus sequences and inserted sequences encoding a *Borrelia burgdorferi* immunogen and a tick saliva protein immunogen.

Pharmaceutical compositions including the live, attenuated chimeric flaviviruses described herein are also included in the invention. Further, the invention includes
5 methods of inducing an immune response to tick-borne encephalitis virus or Langat virus in a subject, involving administering to the subject such a pharmaceutical composition. In various examples, the subject does not have but is at risk of developing infection by tick-borne encephalitis virus or Langat virus. In other examples, the subject is infected with tick-borne encephalitis virus or Langat virus.

10 The invention further includes replication-deficient pseudoinfectious flaviviruses including a flavivirus genome including one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein the flavivirus genome
15 includes yellow fever virus sequences in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis virus or a Langat virus, and sequences encoding the signal sequence between the capsid and pre-membrane proteins of the flavivirus genome include a hybrid of sequences encoding yellow fever virus and tick-borne encephalitis or
20 Langat virus capsid/pre-membrane signal sequences, or a variant thereof. In various examples, the sequences encoding the capsid/pre-membrane signal sequence of the flavivirus genome include yellow fever virus sequences in the 5' region and tick-borne encephalitis or Langat virus sequences in the 3' region.

Further, the invention includes replication-deficient pseudoinfectious flaviviruses
25 including a flavivirus genome including one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein the flavivirus genome includes West Nile virus sequences in which sequences encoding pre-membrane and
30 envelope proteins are replaced with sequences encoding pre-membrane and envelope

proteins of a tick-borne encephalitis or a Langat virus, and the sequences encoding the signal sequence between the capsid and pre-membrane proteins of the flavivirus genome include sequences encoding a tick-borne encephalitis or a Langat virus capsid/pre-membrane signal sequence, or a variant thereof.

5 In addition, the invention includes replication-deficient pseudoinfectious flaviviruses including a flavivirus genome including one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein any capsid (C)
10 and non-structural (NS) proteins in the flavivirus genome are from Langat virus and any pre-membrane (prM) and envelope (E) proteins are from a tick-borne encephalitis virus.

By “replication-deficient pseudoinfectious flavivirus” or “PIV” is meant a flavivirus that is replication-deficient due to a deletion or mutation in the flavivirus genome. The deletion or mutation can be, for example, a deletion of a large sequence,
15 such as most of the capsid protein, as described herein (with the cyclization sequence remaining; see below). In other examples, sequences encoding different proteins (e.g., prM, E, NS1, NS3, and/or NS5; see below) or combinations of proteins (e.g., prM-E or C-prM-E) are deleted. This type of deletion may be advantageous if the PIV is to be used a vector to deliver a heterologous immunogen, as the deletion can permit insertion of
20 sequences that may be, for example, at least up to the size of the deleted sequence. In other examples, the mutation can be, for example, a point mutation, provided that it results in replication deficiency, as discussed above. Because of the deletion or mutation, the genome does not encode all proteins necessary to produce a full flavivirus particle. The missing sequences can be provided in trans by a complementing cell line that is
25 engineered to express the missing sequence (e.g., by use of a replicon; s-PIV; see below), or by co-expression of two replication-deficient genomes in the same cell, where the two replication-deficient genomes, when considered together, encode all proteins necessary for production (d-PIV system; see below).

Upon introduction into cells that do not express complementing proteins, the
30 genomes replicate and, in some instances, generate “virus-like particles,” which are

released from the cells and are able to leave the cells and be immunogenic, but cannot infect other cells and lead to the generation of further particles. For example, in the case of a PIV including a deletion in capsid protein encoding sequences, after infection of cells that do not express capsid, VLPs including prM-E proteins are released from the cells.

- 5 Because of the lack of capsid protein, the VLPs lack capsid and a nucleic acid genome. In the case of the d-PIV approach, production of further PIVs is possible in cells that are infected with two PIVs that complement each other with respect to the production of all required proteins (see below).

- The invention provides several advantages. For example, the PIV vectors and
10 PIVs of the invention are highly attenuated and highly efficacious after one-to-two doses, providing durable immunity. Further, unlike inactivated vaccines, PIVs mimic whole virus infection, which can result in increased efficacy due to the induction of robust B- and T-cell responses, higher durability of immunity, and decreased dose requirements. In addition, similar to whole viruses, PIV vaccines target antigen-presenting cells, such as
15 dendritic cells, stimulate toll-like receptors (TLRs), and induce balanced Th1/Th2 immunity. PIV constructs have also been shown to grow to high titers in substrate cells, with little or no CPE, allowing for high-yield manufacture, optionally employing multiple harvests and/or expansion of infected substrate cells. Further, the PIV vectors of the invention provide an option for developing vaccines against non-flavivirus pathogens for
20 which no vaccines are currently available.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

Brief Description of the Drawings

- 25 Fig. 1 is a schematic illustration of single component PIV (s-PIV) technology.

Fig. 2 is a schematic illustration of two-component PIV (d-PIV) technology.

Fig. 3 is a schematic illustration of a general experimental design for testing immunogenicity and efficacy of PIVs in mice.

- Fig. 4 is a graph comparing the humoral immune response induced by PIV-WN
30 (RV-WN) with that of YF/WN LAV (CV-WN) in mice.

Fig. 5 is a series of graphs showing the results of challenging hamsters immunized with PIV-YF (RV-YF), YF17D, PIV-WN (RV-WN), and YF/WN LAV (CVWN) with hamster-adapted Asibi (PIV-YF and YF17D vaccinees) and wild type WN-NY99 (PIV-WN and YF/WN LAV vaccinees).

5 Fig. 6 is a table showing YF/TBE and YF/LGT virus titers and plaque morphology obtained with the indicated chimeric flaviviruses.

Fig. 7 is a table showing WN/TBE PIV titers and examples of immunofluorescence of cells containing the indicated PIVs.

Fig. 8 is a set of graphs showing the replication kinetics of YF/TBE LAV and PIV-WN/TBE in Vero and BHK cell lines (CV-Hypr = YF/Hypr LAV; CV-LGT = YF/LGT LAV; RV-WN/TBEV = PIV-WN/TBEV).

Fig. 9 is a series of graphs showing survival of mice inoculated IC with PIV-TBE and YF/TBE LAV constructs in a neurovirulence test (3.5 week old ICR mice; RV-WN/Hypr = PIV-WN/TBE(Hypr); CV-Hypr = YF/TBE(Hypr) LAV; CV-LGT = YF/LGT LAV).

Fig. 10 is a graph showing survival of mice inoculated IP with PIV-WN/TBE(Hypr) (RV-WN/Hypr), YF/TBE(Hypr) LAV (CV-Hypr), and YF/LGT LAV (CV-LGT) constructs and YF17D in a neuroinvasiveness test (3.5 week old ICR mice).

Fig. 11 is a series of graphs illustrating morbidity in mice measured by dynamics of body weight loss after TBE virus challenge, for groups immunized with s-PIV-TBE candidates (upper left panel), YF/TBE and YF/LGT chimeric viruses (upper right panel), and controls (YF 17D, human killed TBE vaccine, and mock; bottom panel).

Fig. 12 is a schematic representation of PIV constructs expressing rabies virus G protein, as well as illustration of packaging of the constructs to make pseudoinfectious virus and immunization.

Fig. 13 is a schematic representation of insertion designs resulting in viable/expressing constructs (exemplified by rabies G).

Fig. 14 is series of images showing immunofluorescence analysis and graphs showing growth curves of cells transfected with the indicated PIV-WN constructs (Δ C-Rabies G, Δ PrM-E-Rabies G, and Δ C-PrM-E-Rabies G).

Fig. 15 is a series of images showing immunofluorescence analysis of RabG expressed on the plasma membranes of Vero cells transfected with the indicated PIV constructs (Δ C-Rabies G, Δ PrM-E-Rabies G, and Δ C-PrM-E-Rabies G).

Fig. 16 is a schematic illustration of a PIV-WN-rabies G construct and a series
5 of images showing that this construct spreads in helper cells, but not in naïve cells.

Fig. 17 is a series of graphs showing stability of the rabies G protein gene in PIV-WN vectors.

Fig. 18 is a set of images showing a comparison of spread of single-component vs. two-component PIV-WN-rabies G variants in Vero cells.

10 Fig. 19 is a set of immunofluorescence images showing expression of full-length RSV F protein (strain A2) by the Δ prM-E component of d-PIV-WN in helper cells after transfection.

Detailed Description of the Invention

15 The invention provides replication-defective or deficient pseudoinfectious virus (PIV) vectors including flavivirus sequences, which can be used in methods for inducing immunity against heterologous pathogen, cancer, and allergy-related immunogens inserted into the vectors as well as, optionally, the vectors themselves. The invention also
20 includes compositions including combinations of PIVs and/or PIV vectors, as described herein, and methods of using such compositions to induce immune responses against inserted immunogen sequences and/or sequences of the PIVs themselves. Further, the invention includes particular PIVs and live, attenuated chimeric flaviviruses including tick-borne encephalitis virus sequences, and related vectors, compositions, and methods
25 of use. The PIV vectors, PIVs, live attenuated chimeric flaviviruses, compositions, and methods of the invention are described further below.

PIV Vectors and PIVs

The PIV vectors and PIVs of the invention can be based on the single- or two-
30 component PIVs described above (also see WO 2007/098267 and WO 2008/137163). Thus, for example, in the case of single component PIVs, the PIV vectors and PIVs can

include a genome including a large deletion in capsid protein encoding sequences and be produced in a complementing cell line that produces capsid protein in trans (single component; Fig. 1 and Fig. 12). According to this approach, most of the capsid-encoding region is deleted, which prevents the PIV genome from producing infectious progeny in normal cell lines (i.e., cell lines not expressing capsid sequences) and vaccinated subjects. The capsid deletion typically does not disrupt RNA sequences required for genome cyclization (i.e., the sequence encoding amino acids in the region of positions 1-26), and/or the prM sequence required for maturation of prM to M. In specific examples, the deleted sequences correspond to those encoding amino acids 26-100, 26-93, 31-100, or 31-93 of the C protein.

Single component PIV vectors and PIVs can be propagated in cell lines that express either C or a C-prM-E cassette, where they replicate to high levels. Exemplary cell lines that can be used for expression of single component PIV vectors and PIVs include BHK-21 (e.g., ATCC CCL-10), Vero (e.g., ATCC CCL-81), C7/10, and other cells of vertebrate or mosquito origin. The C or C-prM-E cassette can be expressed in such cells by use of a viral vector-derived replicon, such as an alphavirus replicon (e.g., a replicon based on Venezuelan Equine Encephalitis virus (VEEV), Sindbis virus, Semliki Forest virus (SFV), Eastern Equine Encephalitis virus (EEEV), Western Equine Encephalitis virus (WEEV), or Ross River virus). To decrease the possibility of productive recombination between the PIV vectors/PIVs and complementing sequences, the sequences in the replicons (encoding C, prM, and/or E) can include nucleotide mutations. For example, sequences encoding a complementing C protein can include an unnatural cyclization sequence. The mutations can result from codon optimization, which can provide an additional benefit with respect to PIV yield. Further, in the case of complementing cells expressing C protein sequences (and not a C-prM-E cassette), it may be beneficial to include an anchoring sequence at the carboxy terminus of the C protein including, for example, about 20 amino acids of prM (see, e.g., WO 2007/098267).

The PIV vectors and PIVs of the invention can also be based on the two-component genome technology described above. This technology employs two partial genome constructs, each of which is deficient in expression of at least one protein

required for productive replication (capsid or prM/E) but, when present in the same cell, result in the production of all components necessary to make a PIV. Thus, in one example of the two-component genome technology, the first component includes a large deletion of C, as described above in reference to single component PIVs, and the second component includes a deletion of prM and E (Fig. 2 and Fig. 12). In another example, the first component includes a deletion of C, prM, and E, and the second component includes a deletion of NS1 (Fig. 12). Both components can include cis-acting promoter elements required for RNA replication and a complete set of non-structural proteins, which form the replicative enzyme complex. Thus, both defective genomes can include a 5'-untranslated region and at least about 60 nucleotides (Element 1) of the following, natural protein-coding sequence, which comprises an amino-terminal fragment of the capsid protein. This sequence can be followed by a protease cleavage sequence such as, for example, a ubiquitine or foot-and-mouth disease virus (FAMDV)-specific 2A protease sequence, which can be fused with either capsid or envelope (prM-E) coding sequences. Further, artificial, codon optimized sequences can be used to exclude the possibility of recombination between the two defective viral genomes, which could lead to formation of replication-competent viruses (see, e.g., WO 2008/137163). Use of the two-component genome approach does not require the development of cell lines expressing complementing genomes, such as the cells transformed with replicons, as discussed above in reference to the single component PIV approach. Exemplary cell lines that can be used in the two-component genome approach include Vero (e.g., ATCC CCL-81), BHK-21 (e.g., ATCC CCL-10), C7/10, and other cells of vertebrate or mosquito origin.

Additional examples of d-PIV approaches that can be used in the invention are based on use of complementing genomes including deletions in NS3 or NS5 sequences. A deletion in, e.g., NS1, NS3, or NS5 proteins can be used as long as several hundred amino acids in the ORF, removing the entire chosen protein sequence, or as short as 1 amino acid inactivating protein enzymatic activity (e.g., NS5 RNA polymerase activity, NS3 helicase activity, etc.). Alternatively, point amino acid changes (as few as 1 amino acid mutation, or optionally more mutations) can be introduced into any NS protein, inactivating enzymatic activity. In addition, several Δ NS deletions can be combined in

one helper molecule. The same heterologous gene, i.e., expressed by the first d-PIV component, can be expressed in place or in combination with the NS deletion(s) in the second component, increasing the amount of expressed immunogen. Notably, the insertion capacity of the helper will increase proportionally to the size of NS deletion(s).
5 Alternatively, a different foreign immunogen(s) can be inserted in place of deletion(s) of the helper to produce multivalent vaccines.

Further, additional approaches that can be used in making PIV vectors and PIVs for use in the present invention are described, for example, in WO 99/28487, WO 03/046189, WO 2004/108936, US 2004/0265338, US 2007/0249032, and U.S. Patent
10 No. 7,332,322.

The PIV vectors and PIVs of the invention can be comprised of sequences from a single flavivirus type (e.g., tick-borne encephalitis (TBE, e.g., strain Hypr), Langat (LGT), yellow fever (e.g., YF17D), West Nile, Japanese encephalitis, dengue (serotype 1-4), St. Louis encephalitis, Kunjin, Rocio encephalitis, Ilheus, Central European
15 encephalitis, Siberian encephalitis, Russian Spring-Summer encephalitis, Kyasanur Forest Disease, Omsk Hemorrhagic fever, Louping ill, Powassan, Negishi, Absettarov, Hansalova, and Apoi viruses), or can comprise sequences from two or more different flaviviruses. Sequences of some strains of these viruses are readily available from generally accessible sequence databases; sequences of other strains can be easily
20 determined by methods well known in the art. In the case of PIV vectors and PIVs including sequences of more than one flavivirus, the sequences can be those of a chimeric flavivirus, as described above (also see, e.g., U.S. Patent No. 6,962,708; U.S. Patent No. 6,696,281; and U.S. Patent No. 6,184,024). In certain examples, the chimeras include pre-membrane and envelope sequences from one flavivirus (such as a flavivirus to which
25 immunity may be desired), and capsid and non-structural sequences from a second, different flavivirus. In one specific example, the second flavivirus is a yellow fever virus, such as the vaccine strain YF17D. Other examples include the YF/TBE, YF/LGT, WN/TBE, and WN/LGT chimeras described below. Another example is an LGT/TBE chimera based on LGT virus backbone containing TBE virus prM-E proteins. A PIV
30 vaccine based on this genetic background would have an advantage, because LGT

replicates very efficiently in vitro and is highly attenuated and immunogenic for humans. Thus, a chimeric LGT/TBE PIV vaccine is expected to provide a robust specific immune response in humans against TBE, particularly due to inclusion of TBE prM-E genes.

Vectors of the invention can be based on PIV constructs or live, attenuated
5 chimeric flaviviruses as described herein (in particular, YF/TBE, YF/LGT, WN/TBE, and WN/LGT; see below). Use of PIV constructs as vectors provides particular advantages in certain circumstances, because these constructs by necessity include large deletions, which render the constructs more amenable to accommodation of insertions that are at least up to the size of the deleted sequences, without there being a loss in replication
10 efficiency. Thus, PIV vectors in general can comprise very small insertions (e.g., in the range 6-10, 11-20, 21-100, 101-500, or more amino acid residues combined with the Δ C deletion or other deletions), as well as relatively large insertions or insertions of intermediate size (e.g., in the range 501-1000, 1001-1700, 1701-3000, or 3001-4000 or more residues). In contrast, in certain examples, it may be advantageous to express
15 relatively short sequences in live attenuated viruses, particularly if the insertions are made in the absence of a corresponding deletion. Additional information concerning insertion sites that can be used in the invention is provided below. In addition, as discussed further below, expression of non-flavivirus immunogens in PIVs and chimeric flaviviruses of the invention can result in dual vaccines that elicit protective immunity against both a
20 flavivirus vector virus pathogen and a target heterologous immunogen (e.g., a pathogen (such as a bacterial, viral, parasite, or fungal pathogen), cancer, or allergy-related immunogen).

As discussed above, the PIV vectors and PIVs of the invention can comprise sequences of chimeric flaviviruses, for example, chimeric flaviviruses including pre-
25 membrane and envelope sequences of a first flavivirus (e.g., a flavivirus to which immunity is sought), and capsid and non-structural sequences of a second, different flavivirus, such as a yellow fever virus (e.g., YF17D; see above and also U.S. Patent No. 6,962,708; U.S. Patent No. 6,696,281; and U.S. Patent No. 6,184,024). Further, chimeric flaviviruses of the invention, used as a source for constructing PIVs, or as
30 vaccines/vaccine vectors per se, can optionally include one or more specific attenuating

mutations (e.g., E protein mutations, prM protein mutations, deletions in the C protein, and/or deletions in the 3'UTR), such as any of those described in WO 2006/116182. For example, the C protein or 3'UTR deletions can be directly applied to YF/TBE or YF/LGT chimeras. Similar deletions can be designed and introduced in other chimeric LAV
5 candidates such as based on LGT/TBE, WN/TBE, and WN/LGT genomes. With respect to E protein mutations, attenuating mutations similar to those described for YF/WN chimera in WO 2006/116182 can be designed, e.g., based on the knowledge of crystal structure of the E protein (Rey et al., Nature 375(6529):291-298, 1995), and employed. Further, additional examples of attenuating E protein mutations described for TBE virus
10 and other flaviviruses are provided in Table 9. These can be similarly introduced into chimeric vaccine candidates.

The invention also provides new, particular chimeric flaviviruses, which can be used as a basis for the design of PIV vectors and PIVs, as live attenuated chimeric flavivirus vectors, and as vaccines against the source(s) of the pre-membrane and
15 envelope components of the chimeras. These chimeras include tick-borne encephalitis (TBE) virus or related prM-E sequences. Thus, the chimeras can include prM-E sequences from, for example, the Hypr strain of TBE or Langat (LGT) virus. Capsid and non-structural proteins of the chimeras can include those from yellow fever virus (e.g., YF17D) or West Nile virus (e.g., NY99).

20 A central feature of these exemplary YF/TBE, YF/LGT, WN/TBE, and WN/LGT chimeras is the signal sequence between the capsid and prM proteins. As is shown in the Examples, below, we have found that, in the case of YF-based PIV chimeras, it is advantageous to use a signal sequence comprising yellow fever and TBE sequences (see below). In one example, the signal sequence includes yellow fever sequences in the
25 amino terminal region (e.g., SHDVLTQFLIL) and TBE sequences in the carboxy terminal region (e.g., GMLGMTIA), resulting in the sequence SHDVLTQFLILGMLGMTIA. We have also found that, in the case of WN-based PIV chimeras, it is advantageous to use a signal sequence comprising TBE sequences (e.g., GGTDWMSWLLVIGMLGMTIA). The invention thus includes YF/TBE, YF/LGT,
30 WN/TBE, and WN/LGT chimeras, both PIVs and LAVs, which include the above-noted

signal sequences, or variants thereof having, e.g., 1-8, 2-7, 3-6, or 4-5 amino acid substitutions, deletions, or insertions, which do not substantially interfere with processing at the signal sequence. In various examples, the substitutions are "conservative substitutions," which are characterized by replacement of one amino acid residue with another, biologically similar residue. Examples of conservative substitutions include the substitution of one hydrophobic residue such as isoleucine, valine, leucine, or methionine for another, or the substitution of one polar residue for another, such as between arginine and lysine, between glutamic and aspartic acids, or between glutamine and asparagine and the like. Additional information concerning these chimeras is provided below, in the Examples.

Insertion Sites

Sequences encoding immunogens can be inserted at one or more different sites within the vectors of the invention. Relatively short peptides can be delivered on the surface of PIV or LAV glycoproteins (e.g., prM, E, and/or NS1 proteins) and/or in the context of other proteins (to induce predominantly B-cell and T-cell responses, respectively). Other inserts, including larger portions of foreign proteins, as well as complete proteins, can be expressed intergenically, at the N- and C-termini of the polyprotein, or bicistronically (e.g., within the ORF under an IRES or in the 3'UTR under an IRES; see, e.g., WO 02/102828, WO 2008/036146, WO 2008/094674, WO 2008/100464, WO 2008/115314, and below for further details). In PIV constructs, there is an additional option of inserting a foreign amino acid sequence directly in place of introduced deletion(s). Insertions can be made in, for example, ΔC , $\Delta prM-E$, $\Delta C-prM-E$, $\Delta NS1$, $\Delta NS3$, and $\Delta NS5$. Thus, in one example, in the case of s-PIVs and the ΔC component of d-PIVs, immunogen-encoding sequences can be inserted in place of deleted capsid sequences. Immunogen-encoding sequences can also, optionally, be inserted in place of deleted prM-E sequences in the $\Delta prM-E$ component of d-PIVs. In another example, the sequences are inserted in place of or combined with deleted sequences in $\Delta C-prM-E$ constructs. Examples of such insertions are provided in the Examples section, below.

In the case of making insertions into PIV deletions, the insertions can be made with a few (e.g., 1, 2, 3, 4, or 5) additional vector-specific residues at the N- and/or C-termini of the foreign immunogen, if the sequence is simply fused in-frame (e.g., ~ 20 first a.a. and a few last residues of the C protein if the sequence replaces the Δ C deletion),
5 or without, if the foreign immunogen is flanked by appropriate elements well known in the field (e.g., viral protease cleavage sites; cellular protease cleavage sites, such as signalase, furin, etc.; autoprotease; termination codon; and/or IRES elements).

If a protein is expressed outside of the continuous viral open reading frame (ORF), e.g., if vector and non-vector sequences are separated by an internal ribosome
10 entry site (IRES), cytoplasmic expression of the product can be achieved or the product can be directed towards the secretory pathway by using appropriate signal/anchor segments, as desired. If the protein is expressed within the vector ORF, important considerations include cleavage of the foreign protein from the nascent polyprotein
15 sequence, and maintaining correct topology of the foreign protein and all viral proteins (to ensure vector viability) relative to the ER membrane, e.g., translocation of secreted proteins into the ER lumen, or keeping cytoplasmic proteins or membrane-associated proteins in the cytoplasm/in association with the ER membrane.

In more detail, the above-described approaches to making insertions can employ the use of, for instance, appropriate vector-derived, insert-derived, or unrelated signal and
20 anchor sequences included at the N and C termini of glycoprotein inserts. Standard autoproteases, such as FMDV 2A autoprotease (~20 amino acids) or ubiquitin (gene ~ 500 nt), or flanking viral NS2B/NS3 protease cleavage sites can be used to direct cleavage of an expressed product from a growing polypeptide chain, to release a foreign protein from a vector polyprotein, and to ensure viability of the construct. Optionally,
25 growth of the polyprotein chain can be terminated by using a termination codon, e.g., following a foreign gene insert, and synthesis of the remaining proteins in the constructs can be re-initiated by incorporation of an IRES element, e.g., the encephalomyocarditis virus (EMCV) IRES commonly used in the field of RNA virus vectors. Viable recombinants can be recovered from helper cells (or regular cells for d-PIV versions).
30 Optionally, backbone PIV sequences can be rearranged, e.g., if the latter results in more

efficient expression of a foreign gene. For example, a gene rearrangement has been applied to TBE virus, in which the prM-E genes were moved to the 3' end of the genome under the control of an IRES (Orlinger et al., J. Virol. 80:12197-12208, 2006).

Translocation of prM-E or any other genes can be applied to PIV flavivirus vaccine candidates and expression vectors, according to the invention.

Additional details concerning different insertion sites that can be used in the invention are as follows (also see WO 02/102828, WO 2008/036146, WO 2008/094674, WO 2008/100464, WO 2008/115314, as noted above). Peptide sequences can be inserted within the envelope protein, which is the principle target for neutralizing antibodies. The sequences can be inserted into the envelope in, for example, positions corresponding to amino acid positions 59, 207, 231, 277, 287, 340, and/or 436 of the Japanese encephalitis virus envelope protein (see, e.g., WO 2008/115314 and WO 02/102828). To identify the corresponding loci in different flaviviruses, the flavivirus sequences are aligned with that of Japanese encephalitis virus. As there may not be an exact match, it should be understood that, in non-JE viruses, the site of insertion may vary by, for example, 1, 2, 3, 4, or 5 amino acids, in either direction. Further, given the identification of such sites as being permissive in JE, they can also vary in JE by, for example, 1, 2, 3, 4, or 5 amino acids, in either direction. Additional permissive sites can be identified using methods such as transposon mutagenesis (see, e.g., WO 02/102828 and WO 2008/036146). The insertions can be made at the indicated amino acids by insertion just C-terminal to the indicated amino acids (i.e., between amino acids 51-52, 207-208, 231-232, 277-278, 287-288, 340-341, and 436-437), or in place of short deletions (e.g., deletions of 1, 2, 3, 4, 5, 6, 7, or 8 amino acids) beginning at the indicated amino acids (or within 1-5 positions thereof, in either direction).

In addition to the envelope protein, insertions can be made into other virus proteins including, for example, the membrane/pre-membrane protein and NS1 (see, e.g., WO 2008/036146). For example, insertions can be made into a sequence preceding the capsid/pre-membrane cleavage site (at, e.g., -4, -2, or -1) or within the first 50 amino acids of the pre-membrane protein (e.g., at position 26), and/or between amino acids 236 and 237 of NS1 (or in regions surrounding the indicated sequences, as described above).

In other examples, insertions can be made intergenically. For example, an insertion can be made between E and NS1 proteins and/or between NS2B and NS3 proteins (see, e.g., WO 2008/100464). In one example of an intergenic insertion, the inserted sequence can be fused with the C-terminus of the E protein of the vector, after the C-terminal
5 signal/anchor sequence of the E protein, and the insertion can include a C-terminal anchor/signal sequence, which is fused with vector NS1 sequences. In another example of an intergenic insertion, the inserted sequences, with flanking protease cleavage sites (e.g., YF 17D cleavage sites), can be inserted into a unique restriction site introduced at the NS2B/NS3 junction (WO 2008/100464).

10 In other examples, a sequence can be inserted in the context of an internal ribosome entry site (IRES, e.g., an IRES derived from encephalomyocarditis virus; EMCV), as noted above, such as inserted in the 3'-untranslated region (WO 2008/094674). In one example of such a vector, employing, for example, yellow fever virus sequences, an IRES-immunogen cassette can be inserted into a multiple cloning site
15 engineered into the 3'-untranslated region of the vector, e.g., in a deletion (e.g., a 136 nucleotide deletion in the case of a yellow fever virus-based example) after the polyprotein stop codon (WO 2008/094674).

Details concerning the insertion of rabies virus G protein and full-length respiratory syncytial virus (RSV) F protein into s-PIV and d-PIV vectors of the invention
20 are provided below in Example 3. The information provided in Example 3 can be applied in the context of other vectors and immunogens described herein.

Immunogens

PIVs (s-PIVs and d-PIVs) based on flavivirus sequences and live, attenuated
25 chimeric flaviviruses (e.g., YF/TBE, YF/LGT, WN/TBE, and WN/LGT), as described above, can be used in the invention to deliver foreign (e.g., non-flavivirus) pathogen (e.g., viral, bacterial, fungal, and parasitic pathogens), cancer, and allergy-related immunogens. As discussed further below, in certain examples, it may be advantageous to target several pathogens occupying the same ecological niche, in a particular geographical region.

30 Specific, non-limiting examples of such immunogens are provided as follows.

In addition to TBE virus, ticks are known to transmit another major disease, Lyme disease. Thus, in a first example, PIVs of the invention, such as PIVs including TBE/LGT sequences, as well as chimeric flaviviruses including TBE sequences (e.g., YF/TBE, YF/LGT, WN/TBE, LGT/TBE, and WN/LGT; in all instances where “TBE” is indicated, this includes the option of using the Hypr strain), can be used as vectors to deliver protective immunogens of the causative agent of Lyme disease (tick-borne spirochete *Borrelia burgdorferi*). This combination, targeting both infectious agents (TBE and *B. burgdorferi*) is advantageous, because TBE and Lyme disease are both tick-borne diseases. The PIV approaches can be applied to chimeras (e.g., YF/TBE, YF/LGT, WN/TBE, or WN/LGT), according to the invention, as well as to non-chimeric TBE and LGT viruses. An exemplary immunogen from *B. burgdorferi* that can be used in the invention is OspA (Gipson et al., Vaccine 21:3875-3884, 2003). Optionally, to increase safety and/or immunogenicity, OspA can be mutated to reduce chances of autoimmune responses and/or to eliminate sites for unwanted post-translational modification in vertebrate animal cells, such as N-linked glycosylation, which may affect immunogenicity of the expression product. Mutations that decrease autoimmunity can include, e.g., those described by Willett et al., Proc. Natl. Acad. Sci. U.S.A. 101:1303-1308, 2004. In one example, FTK-OspA, a putative cross-reactive T cell epitope, *Bb* OspA₁₆₅₋₁₇₃ (YVLEGTLTA) is altered to resemble the corresponding peptide sequence of *Borrelia afzelli* (FTLEGKVAN). In FTK-OspA, the corresponding sequence is FTLEGKLTA.

The sequence of OspA is as follows:

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1  mkkyllgigl ilaliackqn vssldeknsv svdlpgemkv lvskeknkdg
kydliatvdk
61  lelkgtsdkn ngsgvlegvk adsksvklti sddlgqttle vfkedgkltv
25  skkvtskdks
121 steekfnek g evsekiitra dgtrleytgi ksdgsgkake vlkgyvlegt
ltaektltlv
181 kegtvtlskn isksgevsve lndtdssaat kktaawnsqt stltitvnsk
ktdlvftke
30  241 ntitvqqyds ngtklegsav eitkldeikn alk

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The full-length sequence and/or immunogenic fragments of the full-length sequence can be used in the present invention. Exemplary fragments can include one or more of domains 1 (amino acids 34-41), 2 (amino acids 65-75), 3 (amino acids 190-220), and 4 (amino acids 250-270) (Jiang et al., Clin. Diag. Lab. Immun. 1(4):406-412, 1994).

Thus, for example, a peptide comprising any one (or more) of the following sequences (which include sequence variations that can be included in the sequence listed above, in any combination) can be delivered: LPGE/GM/IK/T/GVL; GTSDKN/S/DNGSGV/T; N/H/EIS/P/L/A/SK/NSGEV/IS/TV/AE/ALN/DDT/SD/NS/TS/TA/Q/RATKKTA/GA/K/
 5 TWN/DS/AG/N/KT; SN/AGTK/NLEGS/N/K/TAVEIT/KK/TLD/KEI/LKN.

In addition to *B. burgdorferi* immunogens, tick saliva proteins, such as 64TRP, Isac, and Salp20, can be expressed, e.g., to generate a vaccine candidate of trivalent-specificity (TBE+Lyme disease+ticks). Alternatively, tick saliva proteins can be expressed instead of *B. burgdorferi* immunogens in TBE sequence-containing vectors. In
 10 addition, there are many other candidate tick saliva proteins that can be used for tick vector vaccine development according to the invention (Francischetti et al., Insect Biochem. Mol. Biol. 35:1142-1161, 2005). One or more of these immunogens can be expressed in s-PIV-TBE. However, d-PIV-TBE may also be selected, because of its large insertion capacity. In addition to PIV-TBE, other PIV vaccines can be used as vectors,
 15 e.g., to protect from Lyme disease and another flavivirus disease, such as West Nile virus. Expression of these immunogens can be evaluated in cell culture, and immunogenicity/protection examined in available animal models (e.g., as described in Gipson et al., Vaccine 21:3875-3884, 2003; Labuda et al., Pathog. 2(e27):0251-0259, 2006). Immunogens of other pathogens can be similarly expressed, in addition to Lyme
 20 disease and tick immunogens, with the purpose of making multivalent vaccine candidates. Exemplary tick saliva immunogens that can be used in the invention include the following:

64TRP (AF469170)

25 MKAFFVLSLL STAALTNAAR AGRLGSDLDLT FGRVHGNLYA GIERAGPRGY PGLTASIGGE
 VGARLGGRAG VGVSSYGYGY PSWGYPYGGY GGYGGYGGYG GYDQGFSGAY GGYPGYGGY
 YPSGYGGYG GSYGGSYGGY YTPNVRASA GAAA

Isac (AF270496)

30 MRTAFTCALL AISFLGSPCS SSSEGLEQDT IVETTTQONLY ERHYRNHSGL CGAQYRNSSH
 AEAVERNCTLN HLPVNVNATW EGIRHRINKT IPQFVKLICN FTVAMPQEFY LVYMGSDGNS
 DFEEDKESTG TDEDSNTGSS AAKVTEALI IEAEENCTAH ITGWTETPT TLEPTTESQF EAIP

Salp20 (EU008559)

MRTALTCALL AISFLGSPCS SSEGGLEKDS RVETTTONLY ERYRKHPGL CGAQYRNSSH
 AEAVYNCTLS LLPLSVNTTW EGIRHRINKT IPEFVNLICN FTVAMPDQFY LVYMGSGNS
 YSEEDDGKT GSSAAVQVTE QLIQAEENC TAHITGWTE APTTLEPTTE TQFEAIS

5

Additional details concerning the TBE-related PIVs and LAVs are provided in Example 2, below.

The invention further provides PIV and LAV-vectored vaccines against other non-flavivirus pathogens, including vaccines having dual action, eliciting protective immunity against both flavivirus (as specified by the vector envelope proteins) and non-flavivirus pathogens (as specified by expressed immunologic determinant(s)). These are similar to the example of PIV-TBE-Lyme disease-tick vector vaccines described above. As mentioned above, such dual-action vaccines can be developed against a broad range of pathogens by expression of immunogens from, for example, viral, bacterial, fungal, and parasitic pathogens, and immunogens associated with cancer and allergy. As specific non-limiting examples, we describe herein the design and biological properties of PIV vectored-rabies and -respiratory syncytial virus (RSV) vaccine candidates constructed by expression of rabies virus G protein or full-length RSV F protein in place of or in combination with various deletions in one- and two-component PIV vectors (see Example 3, below).

As is demonstrated in the Examples, below, s-PIV constructs may be advantageously used to stably deliver relatively short foreign immunogens (similar to Lyme disease agent OspA protein and tick saliva proteins), because insertions are combined with a relatively short ΔC deletion. Two-component PIV vectors may be advantageously used to stably express relatively large immunogens, such as rabies G protein and RSV F, as the insertions in such vectors are combined with, for example, large $\Delta prM-E$, $\Delta C-prM-E$, and/or $\Delta NS1$ deletions. Some of the d-PIV components can be manufactured and used as vaccines individually, for instance, the PIV-RSV F construct described below containing a $\Delta C-prM-E$ deletion. In this case, the vaccine induces an immune response (e.g., neutralizing antibodies) predominantly against the expressed protein, but not against the flavivirus vector virus pathogen. In other examples of the invention, dual immunity is obtained by having immunity induced both to vector and

insert components. Additionally, because of the large insertion capacity of PIV vectors, and the option of using two-component genomes, PIV vectors offer the opportunity to target several non-flavivirus pathogens simultaneously, e.g., by expressing foreign immunogens from two different non-flavivirus pathogens in the two components of a d-PIV.

In addition to the RSV F protein, rabies G protein, Lyme disease protective immunogens, and tick saliva proteins, as examples of foreign immunogens described above, other foreign immunogens can be expressed to target respective diseases including, for example, influenza virus type A and B immunogens. In these examples, a few short epitopes and/or whole genes of viral particle proteins can be used, such as the M2, HA, and NA genes of influenza A, and/or the NB or BM2 genes of influenza B. Shorter fragments of M2, NB, and BM2, corresponding for instance to M2e, the extracellular fragment of M2, can also be used. In addition, fragments of the HA gene, including epitopes identified as HA0 (23 amino acids in length, corresponding to the cleavage site in HA) can be used. Specific examples of influenza-related sequences that can be used in the invention include PAKLLKERGFFGAIAGFLE (HA0), PAKLLKERGFFGAIAGFLEGSGC (HA0), NNATFNVTNVNPISHIRGS (NBe), MSLLTEVETPIRNEWGCRCNDSSD (M2e), MSLLTEVETPTRNEWECRCSDSSD (M2e), MSLLTEVETLTRNGWGCRCSDSSD (M2e), EVETPTRN (M2e), SLLTEVETPIRNEWGCRCNDSSD (M2e), and SLLTEVETPIRNEWGCR (M2e). Additional M2e sequences that can be used in the invention include sequences from the extracellular domain of BM2 protein of influenza B (consensus MLEPFQ, e.g., LEPFQILSISGC), and the M2e peptide from the H5N1 avian flu (MSLLTEVETLTRNGWGCRCSDSSD).

Other examples of pathogen immunogens that can be delivered in the vectors of the invention include codon-optimized SIV or HIV gag (55 kDa), gp120, gp160, SIV mac239-rev/tat/nef genes or analogs from HIV, and other HIV immunogens; immunogens from HPV viruses, such as HPV16, HPV18, etc., e.g., the capsid protein L1 which self-assembles into HPV-like particles, the capsid protein L2 or its immunodominant portions (e.g., amino acids 1-200, 1-88, or 17-36), the E6 and E7 proteins which are involved in

transforming and immortalizing mammalian cells fused together and appropriately mutated (fusion of the two genes creates a fusion protein, referred to as E6E7Rb⁻, that is about 10-fold less capable of transforming fibroblasts, and mutations of the E7 component at 2 residues renders the resulting fusion protein mutant incapable of inducing transformation (Boursnell et al., Vaccine 14:1485-1494, 1996). Other immunogens include protective immunogens from HCV, CMV, HSV2, viruses, malaria parasite, *Mycobacterium tuberculosis* causing tuberculosis, *C. difficile*, and other nosocomial infections, that are known in the art, as well as fungal pathogens, cancer immunogens, and proteins associated with allergy that can be used as vaccine targets.

Foreign immunogen inserts of the invention can be modified in various ways. For instance, codon optimization is used to increase the level of expression and eliminate long repeats in nucleotide sequences to increase insert stability in the RNA genome of PIV vectors. Immunogenicity can be increased by chimerization of proteins with immunostimulatory moieties well known in the art, such as TLR agonists, stimulatory cytokines, components of complement, heat-shock proteins, etc. (e.g., reviewed in "Immunopotentiators in Modern Vaccines," Schijns and O'Hagan Eds., 2006, Elsevier Academic Press: Amsterdam, Boston).

With respect to construction of dual vaccines against rabies and other flavivirus diseases, other combinations, such as TBE + rabies, YF + rabies, etc., can be of interest both for human and veterinary use in corresponding geographical regions, and thus can be similarly generated. Possible designs of expression constructs are not limited to those described herein. For example deletions and insertions can be modified, genetic elements can be rearranged, or other genetic elements (e.g. non-flavivirus, non-rabies signals for secretion, intracellular transport determinants, inclusion of or fusion with immunostimulatory moieties such as cytokines, TLR agonists such as flagellin, multimerization components such as leucine zipper, and peptides that increase the period of protein circulation in the blood) can be used to facilitate antigen presentation and increase immunogenicity. Further, such designs can be applied to s-PIV and d-PIV vaccine candidates based on vector genomes of other flaviviruses, and expressing

immunogens of other pathogens, e.g., including but not limited to pathogens described in elsewhere herein.

Other examples of PIV and LAV vectors of the invention including combination vaccines such as DEN+Chikungunya virus (CHIKV) and YF+CHIKV. CHIKV, an
5 alphavirus, is endemic in Africa, South East Asia, Indian subcontinent and the Islands, and the Pacific Islands and shares ecological/geographical niches with YF and DEN1-4. It causes serious disease primarily associated with severe pain (arthritis, other symptoms similar to DEN) and long-lasting sequelae in the majority of patients (Simon et al., Med. Clin. North Am. 92:1323-1343, 2008; Seneviratne et al., J. Travel Med. 14:320-325,
10 2007). Other examples of PIV and LAV vectors of the invention include YF+Ebola or DEN+Ebola, which co-circulate in Africa.

Immunogens for the above-noted non-flavivirus pathogens, sequences of which are well known in the art, may include glycoprotein B or a pp65/IE1 fusion protein of CMV (Reap et al., Vaccine 25(42):7441-7449, 2007; and references therein), several TB
15 proteins (reviewed in Skeiky et al., Nat. Rev. Microbiol. 4(6):469-476, 2006), malaria parasite antigens such as RTS,S (a pre-erythrocytic circumsporozoite protein, CSP) and others (e.g., reviewed in Li et al., Vaccine 25(14):2567-2574, 2007), CHIKV envelope proteins E1 and E2 (or the C-E2-E1, E2-E1 cassettes), HCV structural proteins C-E1-E2 forming VLPs (Ezelle et al., J. Virol. 76(23):12325-12334, 2002) or other proteins to
20 induce T-cell responses, Ebola virus glycoprotein GP (Yang et al., Virology 377(2):255-264, 2008).

In addition to the immunogens described above, the vectors described herein may include one or more immunogen(s) derived from or that direct an immune response against one or more viruses (e.g., viral target antigen(s)) including, for example, a dsDNA
25 virus (e.g., adenovirus, herpesvirus, epstein-barr virus, herpes simplex type 1, herpes simplex type 2, human herpes virus simplex type 8, human cytomegalovirus, varicella-zoster virus, poxvirus); ssDNA virus (e.g., parvovirus, papillomavirus (e.g., E1, E2, E3, E4, E5, E6, E7, E8, BPV1, BPV2, BPV3, BPV4, BPV5, and BPV6 (*In Papillomavirus and Human Cancer*, edited by H. Pfister (CRC Press, Inc. 1990))); Lancaster et al., Cancer
30 Metast. Rev. pp. 6653-6664, 1987; Pfister et al., Adv. Cancer Res. 48:113-147, 1987));

dsRNA viruses (e.g., reovirus); (+)ssRNA viruses (e.g., picornavirus, coxsackie virus, hepatitis A virus, poliovirus, togavirus, rubella virus, flavivirus, hepatitis C virus, yellow fever virus, dengue virus, west Nile virus); (-)ssRNA viruses (e.g., orthomyxovirus, influenza virus, rhabdovirus, paramyxovirus, measles virus, mumps virus, parainfluenza virus, rhabdovirus, rabies virus); ssRNA-RT viruses (e.g., retrovirus, human immunodeficiency virus (HIV)); and dsDNA-RT viruses (e.g. hepadnavirus, hepatitis B). Immunogens may also be derived from other viruses not listed above but available to those of skill in the art.

With respect to HIV, immunogens may be selected from any HIV isolate. As is well-known in the art, HIV isolates are now classified into discrete genetic subtypes. HIV-1 is known to comprise at least ten subtypes (A, B, C, D, E, F, G, H, J, and K). HIV-2 is known to include at least five subtypes (A, B, C, D, and E). Subtype B has been associated with the HIV epidemic in homosexual men and intravenous drug users worldwide. Most HIV-1 immunogens, laboratory adapted isolates, reagents and mapped epitopes belong to subtype B. In sub-Saharan Africa, India, and China, areas where the incidence of new HIV infections is high, HIV-1 subtype B accounts for only a small minority of infections, and subtype HIV-1 C appears to be the most common infecting subtype. Thus, in certain embodiments, it may be desirable to select immunogens from HIV-1 subtypes B and/or C. It may be desirable to include immunogens from multiple HIV subtypes (e.g., HIV-1 subtypes B and C, HIV-2 subtypes A and B, or a combination of HIV-1 and HIV-2 subtypes) in a single immunological composition. Suitable HIV immunogens include ENV, GAG, POL, NEF, as well as variants, derivatives, and fusion proteins thereof, for example.

Immunogens may also be derived from or direct an immune response against one or more bacterial species (spp.) (e.g., bacterial target antigen(s)) including, for example, *Bacillus* spp. (e.g., *Bacillus anthracis*), *Bordetella* spp. (e.g., *Bordetella pertussis*), *Borrelia* spp. (e.g., *Borrelia burgdorferi*), *Brucella* spp. (e.g., *Brucella abortus*, *Brucella canis*, *Brucella melitensis*, *Brucella suis*), *Campylobacter* spp. (e.g., *Campylobacter jejuni*), *Chlamydia* spp. (e.g., *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Chlamydia trachomatis*), *Clostridium* spp. (e.g., *Clostridium botulinum*, *Clostridium difficile*,

Clostridium perfringens, Clostridium tetani), Corynebacterium spp. (e.g.,
 Corynebacterium diphtheriae), Enterococcus spp. (e.g., Enterococcus faecalis,
 enterococcus faecum), Escherichia spp. (e.g., Escherichia coli), Francisella spp. (e.g.,
 Francisella tularensis), Haemophilus spp. (e.g., Haemophilus influenza), Helicobacter
 5 spp. (e.g., Helicobacter pylori), Legionella spp. (e.g., Legionella pneumophila),
 Leptospira spp. (e.g., Leptospira interrogans), Listeria spp. (e.g., Listeria monocytogenes),
 Mycobacterium spp. (e.g., Mycobacterium leprae, Mycobacterium tuberculosis),
 Mycoplasma spp. (e.g., Mycoplasma pneumoniae), Neisseria spp. (e.g., Neisseria
 gonorrhea, Neisseria meningitidis), Pseudomonas spp. (e.g., Pseudomonas aeruginosa),
 10 Rickettsia spp. (e.g., Rickettsia rickettsii), Salmonella spp. (e.g., Salmonella typhi,
 Salmonella typhimurium), Shigella spp. (e.g., Shigella sonnei), Staphylococcus spp. (e.g.,
 Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus,
 coagulase negative staphylococcus (e.g., U.S. Patent No. 7,473,762)), Streptococcus spp.
 (e.g., Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes),
 15 Treponema spp. (e.g., Treponema pallidum), Vibrio spp. (e.g., Vibrio cholerae), and
 Yersinia spp. (Yersinia pestis). Immunogens may also be derived from or direct the
 immune response against other bacterial species not listed above but available to those of
 skill in the art.

Immunogens may also be derived from or direct an immune response against one
 20 or more parasitic organisms (spp.) (e.g., parasite target antigen(s)) including, for example,
 Ancylostoma spp. (e.g., A. duodenale), Anisakis spp., Ascaris lumbricoides, Balantidium
 coli, Cestoda spp., Cnidosporidae spp., Clonorchis sinensis, Dicrocoelium dendriticum,
 Dicrocoelium hospes, Diphylobothrium latum, Dracunculus spp., Echinococcus spp.
 (e.g., E. granulosus, E. multilocularis), Entamoeba histolytica, Enterobius vermicularis,
 25 Fasciola spp. (e.g., F. hepatica, F. magna, F. gigantica, F. jacksoni), Fasciolopsis buski,
 Giardia spp. (Giardia lamblia), Gnathostoma spp., Hymenolepis spp. (e.g., H. nana, H.
 diminuta), Leishmania spp., Loa loa, Metorchis spp. (M. conjunctus, M. albidus), Necator
 americanus, Oestroidea spp. (e.g., botfly), Onchocercidae spp., Opisthorchis spp. (e.g., O.
 viverrini, O. felinus, O. guayaquilensis, and O. noverca), Plasmodium spp. (e.g., P.
 30 falciparum), Protofasciola robusta, Parafasciolopsis fasciomorphae, Paragonimus

westermani, *Schistosoma* spp. (e.g., *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. haematobium*), *Spirometra erinaceieuropaei*, *Strongyloides stercoralis*, *Taenia* spp. (e.g., *T. saginata*, *T. solium*), *Toxocara* spp. (e.g., *T. canis*, *T. cati*), *Toxoplasma* spp. (e.g., *T. gondii*), *Trichobilharzia regenti*, *Trichinella spiralis*, *Trichuris trichiura*, *Trombiculidae* spp., *Trypanosoma* spp., *Tunga penetrans*, and/or *Wuchereria bancrofti*. Immunogens may also be derived from or direct the immune response against other parasitic organisms not listed above but available to those of skill in the art.

Immunogens may be derived from or direct the immune response against tumor target antigens (e.g., tumor target antigens). The term tumor target antigen (TA) may include both tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs), where a cancerous cell is the source of the antigen. A TA may be an antigen that is expressed on the surface of a tumor cell in higher amounts than is observed on normal cells or an antigen that is expressed on normal cells during fetal development. A TSA is typically an antigen that is unique to tumor cells and is not expressed on normal cells. TAs are typically classified into five categories according to their expression pattern, function, or genetic origin: cancer-testis (CT) antigens (i.e., MAGE, NY-ESO-1); melanocyte differentiation antigens (e.g., Melan A/MART-1, tyrosinase, gp100); mutational antigens (e.g., MUM-1, p53, CDK-4); overexpressed 'self' antigens (e.g., HER-2/neu, p53); and viral antigens (e.g., HPV, EBV). Suitable TAs include, for example, gp100 (Cox et al., *Science* 264:716-719, 1994), MART-1/Melan A (Kawakami et al., *J. Exp. Med.*, 180:347-352, 1994), gp75 (TRP-1) (Wang et al., *J. Exp. Med.*, 186:1131-1140, 1996), tyrosinase (Wolfel et al., *Eur. J. Immunol.*, 24:759-764, 1994), NY-ESO-1 (WO 98/14464; WO 99/18206), melanoma proteoglycan (Hellstrom et al., *J. Immunol.*, 130:1467-1472, 1983), MAGE family antigens (e.g., MAGE-1, 2, 3, 4, 6, and 12; Van der Bruggen et al., *Science* 254:1643-1647, 1991; U.S. Patent No. 6,235,525), BAGE family antigens (Boel et al., *Immunity* 2:167-175, 1995), GAGE family antigens (e.g., GAGE-1,2; Van den Eynde et al., *J. Exp. Med.* 182:689-698, 1995; U.S. Patent No. 6,013,765), RAGE family antigens (e.g., RAGE-1; Gaugler et al., *Immunogenetics* 44:323-330, 1996; U.S. Patent No. 5,939,526), N-acetylglucosaminyltransferase-V (Guilloux et al., *J. Exp. Med.* 183:1173-1183, 1996), p15 (Robbins et al., *J. Immunol.*

154:5944-5950, 1995), β -catenin (Robbins et al., J. Exp. Med., 183:1185-1192, 1996), MUM-1 (Coulie et al., Proc. Natl. Acad. Sci. U.S.A. 92:7976-7980, 1995), cyclin dependent kinase-4 (CDK4) (Wolfel et al., Science 269:1281-1284, 1995), p21-*ras* (Fossum et al., Int. J. Cancer 56:40-45, 1994), BCR-*abl* (Bocchia et al., Blood 85:2680-2684, 1995), p53 (Theobald et al., Proc. Natl. Acad. Sci. U.S.A. 92:11993-11997, 1995), p185 HER2/neu (erb-B1; Fisk et al., J. Exp. Med., 181:2109-2117, 1995), epidermal growth factor receptor (EGFR) (Harris et al., Breast Cancer Res. Treat, 29:1-2, 1994), carcinoembryonic antigens (CEA) (Kwong et al., J. Natl. Cancer Inst., 85:982-990, 1995) U.S. Patent Nos. 5,756,103; 5,274,087; 5,571,710; 6,071,716; 5,698,530; 6,045,802; EP 263933; EP 346710; and EP 784483; carcinoma-associated mutated mucins (e.g., MUC-1 gene products; Jerome et al., J. Immunol., 151:1654-1662, 1993); EBNA gene products of EBV (e.g., EBNA-1; Rickinson et al., Cancer Surveys 13:53-80, 1992); E7, E6 proteins of human papillomavirus (Ressing et al., J. Immunol. 154:5934-5943, 1995); prostate specific antigen (PSA; Xue et al., The Prostate 30:73-78, 1997); prostate specific membrane antigen (PSMA; Israeli et al., Cancer Res. 54:1807-1811, 1994); idiotypic epitopes or antigens, for example, immunoglobulin idiotypes or T cell receptor idiotypes (Chen et al., J. Immunol. 153:4775-4787, 1994); KSA (U.S. Patent No. 5,348,887), kinesin 2 (Dietz, et al., Biochem. Biophys. Res. Commun. 275(3):731-738, 2000), HIP-55, TGF β -1 anti-apoptotic factor (Toomey et al., Br. J. Biomed. Sci. 58(3):177-183, 2001), tumor protein D52 (Bryne et al., Genomics 35:523-532, 1996), HIFT, NY-BR-1 (WO 01/47959), NY-BR-62, NY-BR-75, NY-BR-85, NY-BR-87, and NY-BR-96 (Scanlan, M. Serologic and Bioinformatic Approaches to the Identification of Human Tumor Antigens, in *Cancer Vaccines 2000*, Cancer Research Institute, New York, NY), and/or pancreatic cancer antigens (e.g., SEQ ID NOs: 1-288 of U.S. Patent No. 7,473,531). Immunogens may also be derived from or direct the immune response against include TAs not listed above but available to one of skill in the art.

In addition to the specific immunogen sequences listed above, the invention also includes the use of analogs of the sequences. Such analogs include sequences that are, for example, at least 80%, 90%, 95%, or 99% identical to the reference sequences, or fragments thereof. The analogs also include fragments of the reference sequences that

include, for example, one or more immunogenic epitopes of the sequences. Further, the analogs include truncations or expansions of the sequences (e.g., insertion of additional/repeat immunodominant/helper epitopes) by, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11-20, etc., amino acids on either or both ends. Truncation may remove immunologically unimportant or interfering sequences, e.g., within known structural/immunologic domains, or between domains; or whole undesired domains can be deleted; such modifications can be in the ranges 21-30, 31-50, 51-100, 101-400, etc. amino acids. The ranges also include, e.g., 20-400, 30-100, and 50-100 amino acids.

10 Cocktails

The invention also includes compositions including mixtures of two or more PIVs and/or PIV vectors, as described herein. As discussed above, use of such mixtures or cocktails may be particularly advantageous when induction of immunity to more than one immunogen and/or pathogen is desired. This may be useful, for example, in vaccination against different flaviviruses that may be endemic to the region in which the vaccine recipient resides. This may also be useful in the context of administration of multiple immunogens against the same target.

Non-limiting examples of PIV cocktails included in the invention are those including PIV-JE + PIV-DEN, and PIV-YF + PIV-DEN. In both of these examples, the PIVs for either or both components can be single or dual component PIVs, as described above. In addition, in the case of the PIV-DEN, the PIV can include sequences of just one dengue serotype selected from the group consisting of dengue serotypes 1-4, or the cocktail can include PIVs expressing sequences from two, three, or all four of the serotypes. Further, the TBE/*Borrelia burgdorferi*/tick saliva protein (e.g., 64TRP, Isac, Salp20) vaccines described herein can be based on including the different immunogens within a single PIV or live attenuated flavivirus, or can be based on mixtures of PIVs (or LAVs), which each include one or more of the immunogens. The cocktails of the invention can be formulated as such or can be mixed just prior to administration.

Use, Formulation, and Administration

The invention includes the PIV vectors, PIVs, LAV vectors, and LAVs, as well as corresponding nucleic acid molecules, pharmaceutical or vaccine compositions, and methods of their use and preparation. The PIV vectors, PIVs, LAV vectors, and LAVs of the invention can be used, for example, in vaccination methods to induce an immune response to TBE or other flavivirus, and/or another expressed immunogen, as described herein. These methods can be prophylactic, in which case they are carried out on subjects (e.g., human subjects or other mammalian subjects) not having, but at risk of developing infection or disease caused by TBE or another flavivirus and/or a pathogen from which the other expressed immunogen is derived. The methods can also be therapeutic, in which they are carried out on subjects already having an infection by one or more of the relevant pathogens. Further, the viruses and vectors can be used individually or in combination with one another or other vaccines. The subjects treated according to the methods of the invention include humans, as well as non-human mammals (e.g., livestock, such as, cattle, pigs, horses, sheep, and goats, and domestic animals, including dogs and cats).

Formulation of the PIV vectors, PIVs, LAV vectors, and LAVs of the invention can be carried out using methods that are standard in the art. Numerous pharmaceutically acceptable solutions for use in vaccine preparation are well known and can readily be adapted for use in the present invention by those of skill in this art (see, e.g., *Remington's Pharmaceutical Sciences* (18th edition), ed. A. Gennaro, 1990, Mack Publishing Co., Easton, PA). In two specific examples, the PIV vectors, PIVs, LAV vectors, and LAVs are formulated in Minimum Essential Medium Earle's Salt (MEME) containing 7.5% lactose and 2.5% human serum albumin or MEME containing 10% sorbitol. However, the PIV vectors, PIVs, LAV vectors, and LAVs can simply be diluted in a physiologically acceptable solution, such as sterile saline or sterile buffered saline.

The PIV vectors, PIVs, LAV vectors, and LAVs of the invention can be administered using methods that are well known in the art, and appropriate amounts of the viruses and vectors to be administered can readily be determined by those of skill in the art. What is determined to be an appropriate amount of virus to administer can be

determined by consideration of factors such as, e.g., the size and general health of the subject to whom the virus is to be administered. For example, in the case of live, attenuated viruses of the invention, the viruses can be formulated as sterile aqueous solutions containing between 10^2 and 10^8 , e.g., 10^3 to 10^7 , infectious units (e.g., plaque-forming units or tissue culture infectious doses) in a dose volume of 0.1 to 1.0 ml. PIVs can be administered at similar doses and in similar volumes; PIV titers however are usually measured in, e.g., focus-forming units determined by immunostaining of foci, as these defective constructs tend not to form virus-like plaques. Doses can range between 10^2 and 10^8 FFU and administered in volumes of 0.1 to 1.0 ml.

All viruses and vectors of the invention can be administered by, for example, intradermal, subcutaneous, intramuscular, intraperitoneal, or oral routes. In specific examples, dendritic cells are targeted by intradermal or transcutaneous administration, by use of, for example, microneedles or microabrasion devices. Further, the vaccines of the invention can be administered in a single dose or, optionally, administration can involve the use of a priming dose followed by a booster dose that is administered, e.g., 2-6 months later, as determined to be appropriate by those of skill in the art. Optionally, PIV vaccines can be administered via DNA or RNA immunization using methods known to those skilled in the art (Chang et al., Nat. Biotechnol. 26:571-577, 2008; Kofler et al., Proc. Natl. Acad. Sci. U.S.A. 101:1951-1956, 2004).

Optionally, adjuvants that are known to those skilled in the art can be used in the administration of the viruses and vectors of the invention. Adjuvants that can be used to enhance the immunogenicity of the viruses include, for example, liposomal formulations, synthetic adjuvants, such as (e.g., QS21), muramyl dipeptide, monophosphoryl lipid A, polyphosphazine, CpG oligonucleotides, or other molecules that appear to work by activating Toll-like Receptor (TLR) molecules on the surface of cells or on nuclear membranes within cells. Although these adjuvants are typically used to enhance immune responses to inactivated vaccines, they can also be used with live or replication-defective vaccines. Both agonists of TLRs or antagonists may be useful in the case of live or replication-defective vaccines. The vaccine candidates can be designed to express TLR agonists. In the case of a virus delivered via a mucosal route, for example, orally,

mucosal adjuvants such as the heat-labile toxin of *E. coli* (LT) or mutant derivations of LT can be used as adjuvants. In addition, genes encoding cytokines that have adjuvant activities can be inserted into the vaccine candidates. Thus, genes encoding desired cytokines, such as GM-CSF, IL-2, IL-12, IL-13, IL-5, etc., can be inserted together with
5 foreign immunogen genes to produce a vaccine that results in enhanced immune responses, or to modulate immunity directed more specifically towards cellular, humoral, or mucosal responses (e.g., reviewed in “Immunopotentiators in Modern Vaccines”, Schijns and O’Hagan Eds., 2006, Elsevier Academic Press: Amsterdam, Boston, etc.). Optionally, a patch containing a layer of an appropriate toxin-derived adjuvant, can be
10 applied over the injection site. Toxin promotes local inflammation attracting lymphocytes, which leads to a more robust immune response.

Examples

Additional details concerning the invention are provided in the Examples, below.

15 In the Examples, experiments are described in which PIVs based on WN, JE, and YF viruses (see, e.g., WO 2007/098267 and WO 2008/137163) were tested. Firstly, we demonstrated that the constructs are significantly more attenuated in a sensitive suckling mouse neurovirulence model (zero mortality at all tested doses) as compared to available LAV controls (YF 17D, YF/JE LAV, and YF/WN LAV). We demonstrated for the first
20 time that d-PIV constructs were avirulent in this model and thus that two-component PIVs do not undergo uncontrolled (unlimited) spread in vivo and cannot cause clinical signs. Secondly, we performed comparisons of the immunogenicity and efficacy of the PIVs and the LAVs, and demonstrated that PIV vaccines can induce immune response comparable to LAVs and be equally efficacious (e.g., as observed for PIV-WN and
25 YF/WN LAV pair of vaccines). In one pair examined, YF 17D LAV was significantly more immunogenic than PIV-YF. Thus, production of VLPs can vary between different, similarly designed PIV constructs. Specifically, we propose that PIV-YF does not generate a large amount of YF VLPs compared to PIV-WN (WN VLPs), and that increased production of VLPs can be achieved by genetic modifications at the C/prM
30 junction in suboptimal PIV constructs. Specifically, the C/prM junction is an important

location in the flavivirus polyprotein orchestrating the formation of viral envelope and synthesis of viral proteins (Yamshchikov and Compans, *Virology* 192:38-51, 1993; Amberg and Rice, *J. Virol.* 73:8083-8094, 1999; Stocks and Lobigs, *J. Virol.* 72:2141-2149, 1998). We propose that secretion of VLPs in PIV infected cells (in contrast to
5 production of viral particles in whole viruses) can be increased by uncoupling of the viral protease and signalase cleavages at the junction, or use of a strong heterologous signal peptide (tPA, etc.) in place of the signal for prM, or by mutagenesis of the signal for prM. The efficiency of signalase cleavage at the C/prM junction of flaviviruses is low (Stocks and Lobigs, *J. Virol.* 72:2141-2149, 1998), e.g., as predicted by SignalP 3.0 on-line
10 program. It is expected that more efficient cleavage efficiency can be achieved by analysis of specific amino acid substitutions near the cleavage site with SignalP 3.0 (e.g., as described in application WO 2008/100464), followed by incorporation of chosen mutation(s) into PIV genomes, recovery of PIV progeny and measuring VLP secretion. Non-flavivirus signals are inserted by methods standard in the art. Uncoupling between
15 the viral protease and signalase cleavages can be achieved by ablating the viral cleavage site by any non-conservative mutation (e.g., RRS in YF17D C to RRA or GRS or RSS, etc.), or deletion of the entire site or some of its 3 residues. If necessary, formation of free N-terminus of the signal of foreign protein can be achieved by using such elements as autoprotease, or termination codon followed by an IRES. Alternatively, the native AUG
20 initiation codon of C can be ablated (in constructs where C protein sequence is unnecessary, e.g., Δ C PIV) and AUG placed in front of foreign gene. Optimization of vector signal can be performed by random mutagenesis, e.g., by insertion of synthetic randomized sequence followed by identification of viable PIV variants with increased VLP secretion.

25 We also discovered that PIV constructs were substantially more immunogenic in hamsters when administered by the IP route, as compared to the subcutaneous route. We concluded that this was most likely due to better targeting of antigen presenting cells in lymphoid tissues, which are abundant in the abdomen, but not abundant in tissues underlying the skin. Based on these observations, we concluded that efficient targeting of
30 PIVs to dendritic cells, abundant in the skin, can be achieved by cutaneous inoculation,

e.g., via skin microabrasion or intradermal injection using microneedles (Dean et al., Hum Vaccin. 1:106-111, 2005).

Further, we have carried out experiments to show the feasibility of administering mixtures, or cocktails, of different PIVs, such as those described herein (e.g., JE+DEN
5 and YF+DEN). In order to administer cocktails, it is important to verify that there is no interference between co-administered components, and that a balanced immune response is induced. Several PIV mixtures were used to immunize rodents and immune responses were compared to PIV constructs administered individually. No interference was observed in mixtures, and thus cocktail PIV vaccines are feasible. Such formulations
10 may be of particular significance in geographical regions where different flaviviruses co-circulate. This could be also used to simultaneously administer several PIV-based vaccines against non-flavivirus pathogens.

Further, we have demonstrated that no neutralizing antibody response is induced against packaging envelope after at least two doses of PIV (and thus antibodies are
15 elicited against VLPs secreted from infected cells). This was demonstrated using the helper (Δ prM-E) component of a d-PIV (see in Fig. 2) packaged individually, or by measuring neutralizing antibodies to heterologous packaging envelopes (e.g., to the WN envelope used to package PIV-JE in helper cells providing WN-specific C-prM-E proteins in trans). The latter observations support sequential use of different PIV
20 vaccines manufactured in a universal helper packaging cells line, and sequential use of different recombinant PIV-vectored vaccines in the same individual, as discussed above. In addition, we confirmed previous observations that PIV constructs can be stably propagated to high yields in vitro, and that no recombination restoring whole virus occurs after prolonged passaging in substrate cells (Mason et al., Virology 351:432-443, 2006;
25 Shustov et al., J. Virol. 21:11737-11748, 2007).

These and other aspects of the invention are further described in the Examples, below.

Example 1. Pseudoinfectious virus platform development studies*Attenuation in suckling mouse neurovirulence (NV) model*

Materials used in the studies described below are described in Table 1 and the
5 references cited therein. These include s-PIV-WN (based on wt WN virus strain NY99
sequences), s-PIV-JE, s-PIV-WN/JE (based on wt WN virus backbone and prM-E genes
from wt JE virus Nakayama strain), s-PIV-YF/WN (YF 17D backbone and prM-E genes
from WN virus), and s-PIV-YF (based on YF 17D sequences). Additional materials
include d-PIV-YF (YF d-PIV, grown in regular BHK cells (Shustov et al., J. Virol.
10 21:11737-11748, 2007), and two-component d-PIV-WN (grown in regular Vero cells;
Suzuki et al., J. Virol. 82:6942-6951, 2008).

Attenuation of these PIV prototypes was compared to LAVs YF 17D, a chimeric
YF/JE virus, and a chimeric YF/WN virus in suckling mouse NV test (IC inoculation)
using highly susceptible 5-day old ICR mice (the chimeric viruses include yellow fever
15 capsid and non-structural sequences, and JE or WN prM-E sequences). None of the
animals that received PIV constructs showed clinical signs or died, while mortality was
observed in animals inoculated with LAVs (Table 2). The YF 17D virus is neurovirulent
for mice of all ages, while the chimeric vaccines are not neurovirulent for adult mice, but
can cause dose-dependent mortality in more sensitive suckling mice (Guirakhoo et al.,
20 Virology 257:363-372, 1999; Arroyo et al., J. Virol. 78:12497-12507, 2004).
Accordingly, 90%-100% of suckling mice that received doses as low as 1 PFU of YF 17D
died. YF/JE and YF/WN LAVs caused partial mortality at much higher doses ($> 2 \log_{10}$
PFU and $3 \log_{10}$ PFU, respectively), with longer average survival time (AST) of animals
that died, as expected. Thus, PIV constructs are completely avirulent in this sensitive
25 model (at least 20,000 - 200,000 times less neurovirulent than the licensed YF 17D
vaccine).

The YF d-PIV and WN d-PIV caused no mortality or clinical signs. Thus, the
two-component PIV variants that theoretically could spread within brain tissue from cells
co-infected by both of their components did not cause disease. Moreover, we tried to
30 detect the d-PIVs in the brains of additional animals in this experiment, sacrificed on day
6 post-inoculation by titration, and detected none (brain tissues from 10 and 11 mice that

received 4 log₁₀ FFU of YF d-PIV and WN d-PIV, respectively, were homogenized and used for titration). Thus, the d-PIVs did not cause spreading infection characteristic of whole virus. YF/JE LAV has been shown to replicate in the brain of adult ICR mice inoculated by the IC route with a peak titer of ~ 6 log₁₀ PFU/g on day 6, albeit without clinical signs (Guirakhoo et al., Virology 257:363-372, 1999). Co-infection of cells with components of a d-PIV is clearly a less efficient process than infection with whole virus. The data show that d-PIV replication in vivo is quickly brought under control by innate immune responses (and adaptive responses in older animals).

10 *Immunogenicity/efficacy in mice and hamsters*

Immunogenicity/efficacy of the PIV prototypes described above was compared to that of chimeric LAV counterparts and YF 17D in mice and Syrian hamsters. The general experiment design is illustrated in Fig. 3 (mice, IP immunization). Experiments in hamsters were performed similarly (plus-minus a few days, SC or IP inoculation with doses indicated below). 3.5-week old ICR mice (for s-PIV-WN and -YF, YF/WN LAV, and YF 17D groups) or C57/BL6 mice (for s-PIV-JE and YF/JE LAV groups) were immunized IP with graded doses of PIV constructs (4-6 log₁₀ FFU/dose) or chimeric LAV and YF 17D LAV controls (4 log₁₀ PFU). Select PIV-WN, -JE and -YF groups were boosted on day 21 with 5 log₁₀ FFU of corresponding constructs (Table 3).

Neutralizing antibody responses were determined in animal sera by standard PRNT₅₀ against YF/WN or /JE LAVs, or YF 17D viruses. PIV-WN induced very high WN-specific neutralizing antibody responses in all groups, with or without boost, as evidenced by PRNT₅₀ titers determined in pools of sera from immunized animals on days 20 and 34, which was comparable to that in the YF/WN LAV control group. Accordingly, animals immunized with both PIV-WN and YF/WN LAV were protected from lethal challenge on day 35 with wt WN virus (IP, 270 LD₅₀), but not mock-immunized animals (Table 3). When WN neutralizing antibodies were measured in sera from individual mice, high uniformity of immune responses was observed (Fig. 4). Thus, single-round PIV vaccines can be as immunogenic and efficacious as corresponding LAVs. PIV-JE was also highly immunogenic (black mice), while immunogenicity of PIV-YF was significantly lower

compared to the YF 17D control (ICR mice). Yet, dose-dependent protection of PIV-YF immunized animals (but not mock-immunized animals) was observed following a severe lethal IC challenge with wt YF strain Asibi virus (500 LD₅₀) (Table 3), which is in agreement with the knowledge that neutralizing antibody titers as low 1:10 are protective against flavivirus infections.

The YF 17D control virus was highly immunogenic (e.g., PRNT₅₀ titer 1:1,280 on day 34), and thus it is able to infect cells and replicate efficiently in vivo, and its envelope is a strong immunogen. Therefore, it is unlikely that low immunogenicity of PIV-YF was due to its inability to infect cells or replicate efficiently in infected cells in vivo. We believe that the low immunogenicity of PIV-YF (e.g., compared to PIV-WN) was most likely due to a low-level production of YF-specific VLPs in PIV-YF infected cells (while VLP secretion is high in PIV-WN infected cells). As discussed above, we propose that immunogenicity of PIV-YF can be significantly increased, e.g., by appropriate modifications at the C/prM junction, e.g., by uncoupling the two protease cleavages that occur at this junction (viral protease and signalase cleavages), and/or by using a strong heterologous signal [e.g., rabies virus G protein signal, or eukaryotic tissue plasminogen activator (tPA) signal (Malin et al., *Microbes and Infection*, 2:1677-1685, 2000), etc.] in place of the YF signal for prM.

A similar experiment was performed in ~ 4.5-week old Syrian hamsters, to compare immunogenicity of PIV constructs to LAV controls in this model. Animals were immunized SC with graded doses of the test articles (Table 4). PIV-WN was highly immunogenic, e.g., WN-specific PRNT₅₀ titers on day 38 (pre-challenge) were 1:320, 1:640, and 1:1280 in groups that received 5, 6, and 6 (prime)+5 (boost) log₁₀ FFU doses, respectively. This was somewhat lower compared to YF/WN LAV 4 log₁₀ PFU control (≥ 1:2560). PIV-JE and -YF induced detectable specific neutralizing antibody responses, albeit with lower titers compared to YF/JE LAV and YF 17D controls. All animals immunized with PIV-WN and YF/WN were solidly protected from lethal challenge with wt WN virus as evidenced by the absence of mortality and morbidity (e.g., loss of body weight after challenge), as well as absence or a significant reduction of postchallenge WN virus viremia. Mock-immunized animals were not protected (Table 4). PIV-JE and -WN

protected animals from respective challenge in dose-dependent fashion. Protective efficacy in this experiment is additionally illustrated in Fig. 5. For example, high post-challenge YF virus (hamster adapted Asibi strain) viremia was observed in mock immunized animals, peaking on day 3 at a titer of $> 8 \log_{10}$ PFU/ml (upper left panel); all of the animals lost weight, and 1 out of 4 died (upper right panel). In contrast, viremia was significantly reduced or absent in hamsters immunized with PIV-YF (two doses; despite relatively low neutralizing titers) or YF 17D; none of these animals lost weight. Similarly, animals immunized with PIV-WN or YF/WN LAV were significantly or completely protected in terms of post-challenge WN virus viremia and body weight loss/mortality, in contrast to mock controls (compare in bottom panels). Thus, high immunogenicity/efficacy of PIV was demonstrated in a second animal model.

In another hamster experiment, animals were immunized with PIV constructs by the IP route, with two doses. Table 5 compares neutralizing immune responses (specific for each vaccine) determined in pooled sera of hamsters in the above-described experiment (SC inoculation) to those after IP immunization, for PIV-WN, - YF/WN, - WN/JE, and -YF after the first dose (days 20-21) and second dose (days 34-38). A clear effect of the immunization route was observed both after the 1st and 2nd doses. For instance, for PIV-WN after 1st dose, SC immunization resulted in WN-specific PRNT50 titer of 1:40, while IP inoculation resulted in much higher titer 1:320 (and after the 2nd dose, titers were similar). A more pronounced effect was observed for other constructs after both the 1st and 2nd doses. Interestingly, PIV-YF/WN was very highly immunogenic by IP route (titer 1:320 after 1st IP dose vs. 1:20 by SC, and 1:1,280 after 2nd dose vs. 1:160 by SC). Similarly, immunogenicity of PIV-JE was significantly increased (e.g., JE-specific titer of 1:640 after two IP poses). Thus, better targeting of lymphoid cells, specifically antigen-presenting cells (which are more abundant in the abdomen as opposed to tissues under the skin), is an important consideration for use of PIV vaccines. In humans, efficient targeting of dendritic cells of the skin, increasing the magnitude of immune response, can be achieved by intradermal delivery, which we thus propose for a route for PIV immunization of humans.

In the above-described experiments, we also determined whether a neutralizing

antibody response was induced against packaging envelopes (as opposed to response to VLPs encoded by PIV constructs and secreted by infected cells). No WN-specific neutralizing antibodies were detected by PRNT₅₀ in animals immunized with 5 log₁₀ FFU of the second component of WN d-PIV, containing the Δ C-prM-E deletion and thus not encoding VLPs, but packaged into the WN envelope in BHK-CprME(WN) helper cells, and no YF-specific neutralizing activity was found in sera from animals immunized with 4 log₁₀ FFU of the second component of YF d-PIV packaged in YF envelope. No YF-specific neutralizing response was induced by two doses of PIV-YF/WN packaged into YF envelope, and similarly, no WN-specific response was induced by two doses of PIV-JE packaged into WN envelope. The absence of neutralizing response against packaging envelopes permits manufacturing different PIV vaccines in one (universal) manufacturing helper cell line, or immunization of one individual with different recombinant vaccines based on the same vector, according to the present invention.

PIV cocktails

Because PIVs undergo a single (optionally several, but limited) round(s) of replication in vivo, we considered that mixtures of different PIV vaccines can be administered without interference between individual constructs in the mixture (cocktail). To elucidate whether PIV vaccines can be used in cocktail formulations, immune responses in mice and hamsters to several PIV constructs given as mixtures were compared to the same constructs given individually. Similar results were obtained in both animal models. Results of mouse experiments are shown in Table 6. Similar anti-JE neutralizing antibody titers were observed in pools of sera from animals that were given one or two doses of either PIV-JE + PIV-WN mixture or PIV-JE alone (1:20 vs. 1:80 and 1:640 vs. 1:160, for one and two doses, respectively). Similarly, WN-specific titers against PIV-JE + PIV-WN mixture and PIV-WN alone were similar (1:320 vs. 1:640 and 1:5,120 vs. 1:5,120 for one and 2 doses, respectively). No or little cross-specific response was induced by either PIV-JE or -WN. The result was also confirmed by measuring PRNT₅₀ titers in sera from individual animals. Thus, it is clear that PIV vaccines can be efficiently administered as cocktails, inducing immunity against two or

more flavivirus pathogens. In addition, as discussed above, various cocktails can be made between non-flavivirus PIV vaccines, or between any of flavivirus and non-flavivirus PIV vaccines.

5 *In vitro studies*

Different PIV prototypes were serially passaged up to 10 times in helper BHK cells, for s-PIVs, or in regular Vero cells, for d-PIVs. Samples harvested after each passage were titrated in Vero cells by immunostaining. Constructs grew to high titers, and no recombination restoring whole virus was observed. For instance, PIV-WN
10 consistently grew to titers 7-8 log₁₀ FFU/ml in BHK-CprME(WN) helper cells (containing a VEE replicon expressing the WN virus C-prM-E proteins), and WN d-PIV grew to titers exceeding 8 log₁₀ FFU/ml in Vero cells, without recombination.

Example 2. PIV-TBE

15 PIV-TBE vaccine candidates can be assembled based entirely on sequences from wt TBE virus or the closely serologically related Langat (LGT) virus (naturally attenuated virus, e.g., wt strain TP-21 or its empirically attenuated variant, strain E5), or based on chimeric sequences containing the backbone (capsid and non-structural sequences) from YF 17D or other flaviviruses, such as WN virus, and the prM-E envelope protein genes
20 from TBE, LGT, or other serologically related flaviviruses from the TBE serocomplex. YF/TBE LAV candidates are constructed based on the backbone from YF 17D and the prM-E genes from TBE or related viruses (e.g., the E5 strain of LGT), similar to other chimeric LAV vaccines.

Construction of PIV-TBE and YF/TBE LAV vaccine prototypes was performed
25 by cloning of appropriate genetic elements into plasmids for PIV-WN (Mason et al., Virology 351:432-443, 2006; Suzuki et al., J. Virol. 82:6942-6951, 2008), or plasmids for chimeric LAVs (e.g., pBSA-AR1, a single-plasmid version of infectious clone of YF/JE LAV; WO 2008/036146), respectively, using standard methods in the art of reverse genetics. The prM-E sequences of TBE virus strain Hypr (GenBank accession number
30 U39292) and LGT strain E5 (GenBank accession number AF253420) were first computer

codon-optimized to conform to the preferential codon usage in the human genome, and to eliminate nucleotide sequence repeats longer than 8 nt to ensure high genetic stability of inserts (if determined to be necessary, further shortening of nt sequence repeats can be performed). The genes were chemically synthesized and cloned into plasmids for PIV-
5 WN and YF/JE LAV, in place of corresponding prM-E genes. Resulting plasmids were in vitro transcribed and appropriate cells (Vero for chimeric viruses, and helper BHK cells for PIV) were transfected with RNA transcripts to generate virus/PIV samples.

YF/TBE LAV constructs

10 In YF/TBE constructs containing either the TBE Hypr (plasmids p42, p45, and p59) or LGT E5 (plasmid P43) prM-E genes, two different types of the C/prM junction were first examined (see in Fig. 6; C/prM junctions only are shown in Sequence Appendix 1, and complete 5'-terminal sequences covering the 5'UTR-C-prM-E-
beginning of NS1 region are shown in Sequence Appendix 2). The p42-derived
15 YF17D/Hypr chimera contained a hybrid YF17D/Hypr signal peptide for the prM protein, while the p45-derived YF17D/Hypr chimera contained a hybrid YF17D/WN signal peptide for prM (Sequence Appendix 1). The former chimeric virus produced very high titers at both P0 (immediately after transfection) and P1 (the next passage in Vero cells), up to 7.9 log₁₀ PFU/ml, which were 0.5 log₁₀ times higher, compared to the latter virus; in
20 addition it formed significantly larger plaques in Vero cells (Fig. 6). Thus, use of TBE-specific residues in the signal peptide for prM conferred a significant growth advantage over the signal containing WN-specific residues. The p43-derived YF17D/LGT chimera had the same prM signal as the p42-derived virus; it also produced very high titers at P0 and P1 passages (up to 8.1 log₁₀ PFU/ml) and formed large plaques. A derivative of the
25 p42-derived virus was also produced from plasmid p59, which contained a strong attenuating mutation characterized previously in the context of a YF/WN LAV vaccine virus, specifically, a 3-a.a. deletion in the YF17D-specific C protein (PSR, residues 40-42 in the beginning of α -Helix I; WO 2006/116182). As expected, the p59 virus grew to lower titers (5.6 and 6.5 log₁₀ PFU/ml at P0 and P1, respectively), and formed small
30 plaques (determined in a separate titration experiment and thus not shown in Fig. 6),

compared to the parent p42-derived chimera. These initial observations of growth properties of YF/TBE LAV prototypes, and correlation of replication in vitro with plaque morphologies, have been confirmed in growth curve experiments (Fig. 8).

5 *PIV-TBE constructs*

PIV-WN/TBE variants were constructed, and packaged PIV samples were derived from plasmids p39 and p40 (Fig. 7; Sequence Appendix 1 for C/prM junction sequences, and Sequence Appendix 3 for complete 5'UTR-ΔC-prM-E-beginning of NS1 sequences). These contained complete Hypr or WN prM signals, respectively. Both PIVs were
10 successfully recovered and propagated in BHK-CprME(WN) or BHK-C(WN) helper cells (Mason et al., Virology 351:432-443, 2006; Widman et al., Vaccine 26:2762-2771, 2008). The P0 and P1 sample titers of the p39 variant were 0.2 – 1.0 log₁₀ times, higher than p40 variant. In addition, Vero cells infected with p39 variant were stained brighter in immunofluorescence assay using a polyclonal TBE-specific antibody, compared to
15 p40, indicative of more efficient replication (Fig. 7). The higher rate of replication of the p39 candidate than p40 candidate was confirmed in a growth curve experiment (Fig. 8). In the latter experiment, both candidates appeared to grow better in the BHK-C(WN) helper cells compared to BHK-CprME(WN), with the p39 variant reaching titer of ~ 7 log₁₀ PFU/ml on day 5 (note that peak titers have not been reached). The discovery of the
20 effect of prM signal on replication rates of both PIV and chimeric LAV vaccine candidates, and head-to-head comparison of different signals to generate the most efficiently replicating and immunogenic (see above) construct, are a distinguishing feature of our approach. As discussed above, the invention also includes the use of other flavivirus signals, including with appropriate mutations, the uncoupling the viral protease and signalase cleavages at the C/prM junction, e.g., by mutating or deleting the viral
25 protease cleavage site at the C-terminus of C preceding the prM signal, the use of strong non-flavivirus signals (e.g., tPA signal, etc.) in place of prM signal, as well as optimization of sequences downstream from the signalase cleavage site.

Other PIV-TBE variants based entirely on wt TBE (Hypr strain) and LGT virus
30 (TP21 wild type strain or attenuated E5 strain), and chimeric YF 17D backbone/prM-E

(TBE or LGT) sequences are also included in the invention. Helper cells providing appropriate C, C-prM-E, etc., proteins (e.g., TBE-specific) for trans-complementation can be constructed by means of stable DNA transfection or through the use of an appropriate vector, e.g., an alphavirus replicon, such as based on VEE strain TC-83, with antibiotic
5 selection of replicon-containing cells. Vero and BHK21 cells can be used in practice of the invention. The former are an approved substrate for human vaccine manufacture; any other cell line acceptable for human and/or veterinary vaccine manufacturing can be also used. In addition to s-PIV constructs, d-PIV constructs can also be assembled. To additionally ascertain safety for vaccinees and the environment, appropriate modifications
10 can be employed, including the use of degenerate codons and complementary mutations in the 5' and 3' CS elements, to minimize chances of recombination that theoretically could result in viable virus. Following construction, all vaccine candidates can be evaluated in vitro for manufacturability/stability, and in vivo for attenuation and immunogenicity/efficacy, in available pre-clinical animal models, such as those used in
15 development and quality control of TBE and YF vaccines.

Neurovirulence and neuroinvasiveness in mice of PIV-TBE and YF/TBE LAV constructs

Young adult ICR mice (~ 3.5 week-old), were inoculated with graded doses of PIV-TBE and YF/TBE LAV candidates by the IC route to measure neurovirulence, or IP
20 route to measure neuroinvasiveness (and later immunogenicity/efficacy). Animals that received 5 log₁₀ FFU of PIV-Hypr (p39 and p40) variants by both routes survived and showed no signs of sickness, similar to mock-inoculated animals (Table 7), and thus PIV-TBE vaccines are completely avirulent. Mice inoculated IC with YF 17D control (1 – 3 log₁₀ PFU) showed dose-dependent mortality, while all animals inoculated IP (5 log₁₀
25 PFU) survived, in accord with the knowledge that YF 17D virus is not neuroinvasive. All animals that received graded IC doses (2 – 4 log₁₀ PFU) of YF/TBE LAV prototypes p42, p45, p43, and p59 died (moribund animals were humanely euthanized). These variants appear to be less attenuated than YF 17D, e.g., as evidenced by complete mortality and shorter AST at the 2 log₁₀ PFU dose, the lowest dose tested for YF/TBE LAV candidates.
30 The non-neurovirulent phenotype of PIV-TBE, virulent phenotype of YF/TBE LAV and

intermediate-virulence phenotype of YF 17D are also illustrated in Fig. 9, showing survival curves of mice after IC inoculation. It should be noted that the p43 (LGT prM-E genes) and p59 (the dC2 deletion variant of YF/Hypr LAV) were less neurovirulent than p42 and p45 YF/Hypr LAV constructs as evidenced by larger AST values for
 5 corresponding doses (Table 7). In addition, p43 and p59 candidates were non-neuroinvasive, while p42 and p45 caused partial mortality after IP inoculation (5 log₁₀ PFU/dose) (Table 7; Fig. 10). It should be noted however that all the YF/TBE LAV constructs were significantly attenuated as compared to wt TBE viruses, e.g., compared to wt TBE Hypr virus, which is uniformly highly virulent for mice, both at very low IC
 10 (LD₅₀ ~ 0.1 PFU) and IP (LD₅₀ ≤ 10 PFU) doses (Wallner et al., J. Gen. Virol. 77:1035-1042, 1996; Mandl et al., J. Virol. 72:2132-2140, 1998; Mandl et al., J. Gen. Virol. 78:1049-1057, 1997

Immunogenicity/efficacy of PIV-TBE and YF/TBE LAV constructs in mice

15 TBE-specific neutralizing antibody responses in mice immunized IP with one or two doses of the PIV-TBE or YF/TBE LAV variants described above, or a human formalin-inactivated TBE vaccine control (1:30 of human dose) are being measured. Animals have been challenged with a high IP dose (500 PFU) of wt Hypr TBE virus; morbidity (e.g., weight loss), and mortality after challenge are monitored.

20

Immunogenicity/efficacy of PIV-TBE and YF/TBE LAV constructs in mice

TBE-specific neutralizing antibody responses in mice immunized IP with one or two doses of the PIV-TBE or YF/TBE LAV variants described above (from experiment in Table 7), or a human formalin-inactivated TBE vaccine control (1:20 of human dose;
 25 one or two doses), or YF 17D and mock controls, were measured on day 20 by PRNT₅₀ against wt TBE Hypr virus (Table 8; second dose of indicated test articles was given on day 14). [Titers were determined in individual sera, or pooled sera from two animals in most cases, or pooled sera from 4 animals for the YF17D and Mock negative controls]. Titers in individual test samples as well as GMTs for each group are provided in Table 8.
 30 Titers in test samples were similar within each group, e.g., in groups immunized with

PIVs, indicating high uniformity of immune response in animals. As expected, no TBE-specific neutralizing antibodies were detected in negative control groups (YF 17D and Mock; GMTs < 1:10); accordingly, animals in these groups were not protected from challenge on day 21 post-immunization with a high IP dose (500 PFU) of wt Hypr TBE virus. Mortalities from partial observation (on day 9 post-challenge; observation being continued) are provided in Table 8, and dynamics of average post-challenge body weights indicative of morbidity are shown in Fig. 11. Neutralizing antibodies were detected in killed vaccine controls, which were particularly high after two doses (GMT 1:1,496); animals in the 2-dose group were completely protected in that there was no mortality or body weight loss (but not animals in the 1-dose group). Animals that received both one and two doses of PIV-Hypr p39 had very high antibody titers (GMTs 1:665 and 1:10,584) and were solidly protected, demonstrating that robust protective immunity can be induced by s-PIV-TBE defective vaccine. The two animals that survived immunization with YF/Hypr p42 chimera (see in Table 7) also had high antibody titers (GMT 1:6,085) and were protected (Table 8; Fig. 11). Interestingly, PIV-Hypr p40 and YF/Hypr p45 were poorly immunogenic (GMTs 1:15 and 1:153 for one and two doses, and 1:68, respectively). As discussed above, these contained WN-specific sequences in the signal for prM, while the highly immunogenic PIV-Hypr p39 and YF/Hypr p42 constructs contained TBE-specific signal sequences. In agreement with discussion above, this result demonstrates the importance of choosing the right prM signal, e.g., the TBE-specific signal, to achieve high-level replication/VLP secretion, which in this experiment in vivo resulted in drastically different immune responses. Immunogenicity of YF/LGT p43 and YF/Hypr dC2 p59 chimeras was relatively low which could be expected, because of the use of a heterologous envelope (LGT, different from challenge TBE virus) and high attenuating effect of the dC2 deletion, respectively.

Example 3. Foreign gene expression

In the examples of recombinant PIV constructs described below, genes of interest were codon optimized (e.g., for efficient expression in a target vaccination host) and to eliminate long nt sequence repeats to increase insert stability (≥ 8 nt long;

additional shortening of repeats can be performed if necessary), and then chemically synthesized. The genes were cloned into PIV-WN vector plasmids using standard methods of molecular biology well known in the art, and packaged PIVs were recovered following in vitro transcription and transfection of appropriate helper (for s-PIVs) or
5 regular (for d-PIVs) cells.

Expression of rabies virus G protein in WN s-PIV and d-PIV

Rabies virus, *Rhabdoviridae* family, is a significant human and veterinary pathogen. Despite the availability of several (killed) vaccines, improved vaccines are still
10 needed for both veterinary and human use (e.g. as an inexpensive pre-exposure prophylactic vaccines). Rabies virus glycoprotein G mediates entry of the virus into cells and is the main immunogen. It has been expressed in other vectors with the purpose of developing veterinary vaccines (e.g., Pastoret and Brochier, *Epidemiol. Infect.* 116:235-240, 1996; Li et al., *Virology* 356:147-154, 2006).

15 Full length rabies virus G protein (original Pasteur virus isolate, GenBank accession number NC_001542) was codon-optimized, chemically synthesized, and inserted adjacent to the ΔC , $\Delta prM-E$ and $\Delta C-prM-E$ deletions in PIV-WN vectors (Fig. 12). The sequences of constructs are provided in Sequence Appendix 4. General designs of the constructs are illustrated in Fig. 13. The entire G protein containing its own signal
20 peptide was inserted in-frame downstream from the WN C protein either with the ΔC deletion (ΔC and $\Delta C-prM-E$ constructs) or without ($\Delta prM-E$) and a few residues from the prM signal. Foot and mouth disease virus (FMDV) 2A autoprotease was placed downstream from the transmembrane C-terminal anchor of G to provide cleavage of C-terminus of G from the viral polyprotein during translation. The FMDV 2A element is
25 followed by WN-specific signal for prM and prM-E-NS1-5 genes in the ΔC construct, or signal for NS1 and NS1-5 genes in $\Delta prM-E$ and $\Delta C-prM-E$ constructs.

Packaged WN(ΔC)-rabiesG, WN($\Delta prME$)-rabiesG, and WN($\Delta CprME$)-rabiesG PIVs were produced by transfection of helper BHK cells complementing the PIV vector deletion [containing a Venezuelan equine encephalitis virus (strain TC-83) replicon expressing WN
30 virus structural proteins for trans-complementation]. Efficient replication and expression of

rabies G protein was demonstrated for the three constructs by transfection/infection of BHK-C(WN) and/or BHK-C-prM-E(WN) helper cells, as well as regular BHK cells, by immunostaining and immunofluorescence assay (IFA) using anti-Rabies G monoclonal antibody (RabG-Mab) (Fig. 14). Titers were determined in Vero cells by immunostaining with the Mab or an anti-WN virus polyclonal antibody. Growth curves of the constructs in BHK-CprME(WN) cells after transfection with in vitro RNA transcripts are shown in Fig. 14, bottom panels. The PIVs grew efficiently to titers ~ 6 to $>7 \log_{10}$ FFU/ml. Importantly, nearly identical titers were detected by both RabG-Mab and WN-antibody staining, which was the first evidence of genetic stability of the insert. In PIV-infected Vero cells, which were fixed but not permeabilized, strong membrane staining was observed by RabG-Mab staining, demonstrating that the product was efficiently delivered to the cell surface (Fig. 15). The latter is known to be the main prerequisite for high immunogenicity of expressed G. Individual packaged PIVs can spread following infection of helper BHK cells, but cannot spread in regular cells as illustrated for WN(Δ C)-rabiesG PIV in Fig. 16. The fact that there is no spread in naïve BHK cells demonstrates that the recombinant RNA genomes cannot be non-specifically packaged into membrane vesicles containing the G protein, if produced by PIV infected cells. An identical result was obtained with the G protein of another rhabdovirus, Vesicular stomatitis virus (VSV), contrary to previous observations of non-specific packaging of Semliki Forest virus (SFV) replicon expressing VSV G protein (Rolls et al., Cell 79:497- 506, 1994). The latter is a desired safety feature. [Alternatively, some non-specific packaging could result in a limited spread of PIV in vivo, potentially enhancing anti-rabies immune response. The latter could be also a beneficial feature, given that such PIV is demonstrated to be safe]. The stability of the rabies G insert in the three PIVs was demonstrated by serial passages in helper BHK-CprME(WN) cells at high or low MOI (0.1 or 0.001 FFU/cell). At each passage, cell supernatants were harvested and titrated in regular cells (e.g., Vero cells) using immunostaining with an anti-WN polyclonal antibody to determine total PIV titer, or anti-rabies G monoclonal antibody to determine titer of particles containing the G gene (illustrated for MOI 0.1 in Fig. 17; similar results were obtained at MOI 0.001). The WN(Δ C)-rabiesG PIV was stable for 5 passages, while the titer of insert-containing PIV started declining at passage 6, indicative of insert instability. This could be

expected, because in this construct, large G gene insert (~ 1500 nt) is combined with a small Δ C deletion (~ 200 nt), significantly increasing the overall size of the recombinant RNA genome. In contrast, in WN(Δ prME)-rabiesG, and WN(Δ CprME)-rabiesG PIVs, the insert is combined with a much larger deletion (~ 2000 nt). Therefore, these constructs stably
5 maintained the insert for all 10 passages examined (Fig. 17). Further, it can be seen in Fig. 17 that at some passages, titers as high as 8 log₁₀ FFU/ml, or higher, were attained for all three PIVs, additionally demonstrating that PIVs can be easily propagated to high yields.

Following inoculation in vivo individually, the WN(Δ C)-rabiesG s-PIV is expected to induce strong neutralizing antibody immune responses against both rabies and WN viruses,
10 as well as T-cell responses. The WN(Δ prME)-rabiesG and WN(Δ CprME)-rabiesG PIVs will induce humoral immune response only against rabies because they do not encode the WN prM-E genes. WN(Δ C)-rabiesG s-PIV construct can be also co-inoculated with WN(Δ prME)-rabiesG construct in a d-PIV formulation (see in Fig. 12), increasing the dose of expressed G protein, and with enhanced immunity against both pathogens due to limited
15 spread. As an example of spread, titration results in Vero cells of a s-PIV sample, WN(Δ prME)-rabiesG, and a d-PIV sample, WN(Δ prME)-rabiesG + WN(Δ C) PIV (the latter did not encode rabies G protein), are shown in Fig. 18. Infection of naïve Vero cells with s-PIV gave only individual cells stainable with RabG-Mab (or small clusters formed due to division of cells). In contrast, large foci were observed following infection with the d-PIV
20 sample (Fig. 18, right panel) that were products of coinfection with the two PIV types.

The WN(Δ CprME)-rabiesG construct can be also used in a d-PIV formulation, if it is co-inoculated with a helper genome providing C-prM-E in trans (see in Fig. 12). For example it can be a WN virus genome containing a deletion of one of the NS proteins, e.g., NS1, NS3, or NS5, which are known to be trans-complementable (Khromykh et al., J. Virol.
25 73:10272-10280, 1999; Khromykh et al., J. Virol. 74:3253-3263, 2000). We have constructed a WN- Δ NS1 genome (sequence provided in Sequence Appendix 4) and obtained evidence of co-infection with WN(Δ prME)-rabiesG or WN(Δ CprME)-rabiesG constructs, and spread in vitro, by immunostaining. In the case of such d-PIVs, rabies G protein can be also inserted and expressed in helper genome, e.g., WN- Δ NS1 genome, to increase the
30 amount of expressed rabies G protein resulting in an increased anti-rabies immune response.

As with any dPIV versions, one immunogen can be from one pathogen (e.g., rabies G) and the other from a second pathogen, resulting in three antigenic specificities of vaccine. As discussed above, Δ NS1 deletions can be replaced with or used in combination with Δ NS3 and/or Δ NS5 deletions/mutations, in other examples.

5

Expression of RSV F protein in WN s-PIV and d-PIV

Respiratory syncytial virus (RSV), member of *Paramyxoviridae* family, is the leading cause of severe respiratory tract disease in young children worldwide (Collins and Crowe, Respiratory Syncytial Virus and Metapneumovirus, In: Knipe et al. Eds., Fields Virology, 5th ed., Philadelphia: Wolters Kluwer/Lippincott Williams and Wilkins, 2007:1601-1646).
 10 Fusion protein F of the virus is a lead viral antigen for developing a safe and effective vaccine. To avoid post-vaccination exacerbation of RSV infection observed previously with a formalin-inactivated vaccine candidate, a balanced Th1/Th2 response to F is required which can be achieved by better TLR stimulation, a prerequisite for induction of high-affinity
 15 antibodies (Delgado et al., Nat. Med. 15:34-41, 2009), which should be achievable through delivering F in a robust virus-based vector. We have previously demonstrated the capacity of yellow fever virus-based chimeric LAV vectors to induce a strong, balanced Th1/Th2 response in vivo against an influenza antigen (WO 2008/036146). In the present invention, both yellow fever virus-based chimeric LAVs and PIV vectors are used for delivering RSV F
 20 to induce optimal immune response profile. Other LAVs and PIV vectors described herein can also be used for this purpose.

Full-length RSV F protein of A2 strain of the virus (GenBank accession number P03420) was codon optimized as described above, synthesized, and cloned into plasmids for PIV-WN s-PIV and d-PIV, using the insertion schemes shown in Fig. 12 and 13 for rabies G
 25 protein, by applying standard methods of molecular biology. Exact sequences of the insertions and surrounding genetic elements are provided in Sequence Appendix 5. In vitro RNA transcripts of resulting WN(Δ C)-RSV F, WN(Δ prME)- RSV F, and WN(Δ CprME)- RSV F PIV constructs were used to transfect helper BHK-CprME(WN) cells. Efficient replication and expression of RSV F protein was first demonstrated by immunostaining of
 30 transfected cells with an anti-RSV F Mab, as illustrated for the WN(Δ prME)- RSV F

construct in Fig. 19. The presence of packaged PIVs in the supernatants from transfected cells (titer as high as 7 log₁₀ FFU/ml) was determined by titration in Vero cells with immunostaining. Additionally, similar constructs can be used that contain a modified full length F protein gene. Specifically, the N-terminal native signal peptide of F is replaced in
5 modified F protein with the one from rabies virus G protein. The modification is intended to elucidate whether the use of a heterologous signal can increase the rate of F protein synthesis and/or replication of PIVs.

Table 1. PIV prototype constructs used in platform development studies

Construct	Genetic composition	Packaged in
PIV-WN	wt NY99 WN virus	WN envelope; BHK-CprME(WN) or BHK-C(WN) helper cells (Mason et al., Virology 2006, 351:432-43; Widman et al., Vaccine 2008, 26:2762-71)
PIV-YF/WN	Envelope (VLP): wt WN NY99 Backbone: YF 17D	YF 17D envelope; BHK-CprME(YF) helper cells (Widman et al., Adv Virus Res. 2008, 72:77-126)
PIV-WN/JE	Envelope (VLP): wt JE Nakayama Backbone: wt WN NY99	JE or WN envelope; BHK-C(WN) or BHK-CprME(WN) helper cells (Ishikawa et al., Vaccine 2008, 26:2772-8)
PIV-YF	YF 17D	YF 17D envelope; BHK-CprME(YF) or BHK-C(YF) helper cells (Mason et al., Virology 2006, 351:432-43)

10

Table 2. Safety: Suckling mouse neurovirulence¹

Construct	Doses (log ₁₀)	Mortality (%)	AST (days) ²
PIV-YF	1 - 4	0/10 (0%)	na
PIV-WN	2 - 5	0/10 (0%)	na
PIV-WN/JE	1 - 4	0/11 (0%)	na
PIV-YF/WN	1 - 4	0/10-11 (0%)	na
WN d-PIV	1 - 4	0/10-11 (0%)	na
YF d-PIV	1 - 4	0/10 (0%)	na
YF17D	2	10/10 (100%)	7.6
	1	10/10 (100%)	9.3
	0	9/10 (90%)	9.9
	-1	3/10 (30%)	9.6

YF/JE	4	9/11 (82%)	9.7
	3	7/10 (70%)	12.3
	2	3/11 (27%)	12
	1	0/11 (0%)	na
YF/WN	3	2/11 (18%)	12.5
	0-2	0/10-11 (0%)	na

¹Single dose, IC inoculation, ICR 5-day old mice, graded log doses administered.

²AST for mice that died; na, not applicable.

5

10 Table 3. PIV highly immunogenic and efficacious in mice¹

Group	Dose	PRNT Day 20	PRNT Day 34	Post-challenge mortality (%)
PIV-WN	10 ⁵	640	1280	0/8 (0%)
	10 ⁶	1280	2560	1/8 (12.5%)
	10 ⁶ +10 ⁵	2560	2560	0/6 (0%)
YF/WN control	10 ⁴	1280	2560	1/8 (12.5%)
PIV-WN/JE	10 ⁴	10	20	N/D
	10 ⁵	20	20	N/D
	10 ⁵ +10 ⁵	20	160	N/D
YF/JE control	10 ⁴	160	320	N/D
PIV-YF	10 ⁴	<10	<10	8/8 (100%)
	10 ⁵	<10	<10	5/7 (71%)
	10 ⁵ +10 ⁵	10	10	2/5 (40%)
YF17D control	10 ⁴	640	1280	0/7 (0%)
Mock control – WN challenge	Diluent	N/D	0	7/7 (100%)
– YF challenge	Diluent	N/D	0	8/8 (100%)

¹IP immunization (d0 prime, and d21 boost in select groups); challenge on d35: wt WN NY99, 3 log₁₀ PFU IP, 270 LD50; wt YF Asibi, 3 log₁₀ PFU IC, 500 LD50; N/D, not determined.

15

Table 4. PIV are immunogenic in hamsters and protect against challenge¹

Group	Dose(s)	PRNT Day 38	POST-CHALLENGE		
			Mortality	Morbidity	Peak viremia (log)
PIV-WN	10 ⁵	320	0/5 (0%)	0/5 (0%)	2.3
	10 ⁶	640	0/5 (0%)	0/5 (0%)	1.8
	10 ⁶ +10 ⁵	1280	0/5 (0%)	0/5 (0%)	<1.3
YF/WN control	10 ⁴	≥2560	0/5 (0%)	0/5 (0%)	<1.3
PIV-WN/JE	10 ⁴	20	2/5 (40%)	2/5 (40%)	2.2
	10 ⁵ +10 ⁵	40	0/5 (0%)	0/5 (0%)	<1.3
YF/JE control	10 ⁴	2560	0/5 (0%)	0/5 (0%)	1.3
PIV-YF	10 ⁴	<10	1/3 (33%)	3/3 (100%)	8.3
	10 ⁵	<10	1/5 (20%)	4/5 (80%)	8.3
	10 ⁵ +10 ⁵	20	0/4 (0%)	0/4 (0%)	2.5
YF17D control	10 ⁴	≥2560	0/4 (0%)	0/4 (0%)	<1.3
Mock control – WN challenge	Diluent	<10	3/4 (75%)	4/4 (100%)	4.0
– YF challenge	Diluent	<10	1/4 (25%)	4/4 (100%)	8.4
– JE challenge	Diluent	<10	2/5 (40%)	2/5 (40%)	3.0

¹Syrian hamsters, SC inoculation (d0, and d21 in select groups); challenge (d39): wt WN NY385/99 6 log₁₀

- 5 PFU IP, wt JE Nakayama 5.8 log₁₀ PFU IC, or hamster-adapted YF Asibi 7 log₁₀ PFU IP (McArthur et al., J. Virol. 77:1462-1468, 2003; McArthur et al., Virus Res. 110:65-71, 2005).

5 Table 5. Immunization of hamsters with PIV: comparison of SC and IP routes

Inoculums	PRNT Day 20-21		Boost (log ₁₀)	PRNT Day 34-38	
	SC	IP		SC	IP
PIV-WN	40	320	5	1280	1280
PIV-YF/WN	10	320	5	160	1280
PIV-WN/JE	10	80	5	40	640
PIV-YF	<10	10	5	20	80

10

Table 6. Immune responses to PIV cocktails (mice)¹

Group	Dose	PRNT Day 20		PRNT Day 34	
		Anti-JE	Anti-WN	Anti-JE	Anti-WN
PIV-WN/JE + RV-WN	10 ⁵ + 10 ⁵	20	320	640	5120
PIV-WN/JE alone	10 ⁵	80	<10	160	20
PIV-WN alone	10 ⁵	<10	640	<10	5120
Mock	-	<10	<10	<10	<10

¹ C57/BL6 mice, IP inoculations on days 0 and 21; pooled serum PRNT titers.

15

Table 7. Neurovirulence (IC inoculation) and neuroinvasiveness (IP inoculation) of PIV-TBE and YF/TBE vaccine constructs in adult ICR mice

Construct	Neurovirulence (IC route)			Neuroinvasiveness (IP route)		
	Dose(s) (log ₁₀)	Mortality (%)	AST, days ¹	Dose(s) (log ₁₀)	Mortality (%)	AST, days ¹
PIV-Hypr p39	5	0/7 (0%)	na	5	0/16 (0%)	na
PIV-Hypr p40	5	0/6 (0%)	na	5	0/16 (0%)	na
YF/Hypr p42	4	8/8 (100%)	6.3	5	6/8 (75%)	13.3
	3	8/8 (100%)	6.4			
	2	8/8 (100%)	7.4			
YF/LGT p43	4	8/8 (100%)	7.9	5	0/8 (0%)	na
	3	8/8 (100%)	7.6			
	2	8/8 (100%)	8.4			
YF/Hypr p45	4	8/8 (100%)	6.1	5	5/8 (62.5%)	11.2
	3	8/8 (100%)	6.6			
	2	8/8 (100%)	6.8			
YF/Hypr dC2 p59	4	8/8 (100%)	6.6	5	0/8 (0%)	na
	3	8/8 (100%)	7.4			
	2	8/8 (100%)	8.1			
YF 17D	3	8/8 (100%)	9	5	0/8 (0%)	na
	2	7/8 (87.5%)	9.6			
	1	4/8 (50%)	10			
Mock (diluent)	none	0/8 (0%)	na	none	0/8 (0%)	na

¹AST for mice that died.

5

Table 8. Neutralizing antibody titers (PRNT₅₀) in mice immunized IP (determined against wt TBE virus Hypr), and protection from challenge (postchallenge observation, day 9)

Immunogen	Dose(s), log ₁₀	PRNT ₅₀ titer, individ. samples ¹	PRNT ₅₀ GMT	Postchallenge mortality (%) on day 9 ²
PIV-Hypr p39, 1 dose	5	1746 (2) 1187 (2) 164 (2) 574 (2)	665	0/8 (0%)
PIV-Hypr p39, 2 doses	5+5	16229 (2) 12928 (2) 12927 (2) 4627 (2)	10,584	0/8 (0%)
PIV-Hypr p40, 1 dose	5	<10 (2) <10 (2) 18 (2) 33 (2)	15	6/8 (75%)
PIV-Hypr p40, 2 doses	5+5	169 (2) 638 (2) 26 (2) 192 (2)	153	1/8 (12.5%)
YF/Hypr p42	5	9210 (1) 4020 (1)	6,085	0/2 (0%)
YF/LGT p43	5	123 (2)	64	1/8 (12.5%)

		32 (2)		
		96 (2)		
		45 (2)		
YF/Hypr p45	5	292 (2)	68	0/3 (0%)
		16 (1)		
YF/Hypr dC2 p59	5	194 (2)	68	0/8 (0%)
		93 (2)		
		45 (2)		
		26 (2)		
Killed human TBE vaccine, 1 dose (at 1/20 of human dose)	1/20	19 (2)	12	1/8 (12.5%)
		<10 (2)		
		13 (2)		
		<10 (2)		
Killed human TBE vaccine, 2 doses (each at 1/20 of human dose)	1/20+1/20	3435 (2)	1,496	0/6 (0%)
		1267 (2)		
		770 (2)		
YF 17D control	5	<10 (4)	<10	5/8 (62.5%)
		11 (4)		
Mock	none	<10 (4)	<10	4/8 (50%)
		<10 (4)		

¹Numbers in parenthesis correspond to number of mice in each pooled serum sample tested.

²Mortalities on day 9 are shown.

5

Table 9. Examples of published attenuating E protein mutations that can be used for attenuation of chimeric TBE LAV candidates

Residue	Domain	Comments	Attenuation in	Reference
N52R	II	DI- DII hinge, possibly involved in hinge motion required for fusion activation	JE, YF	Hasegawa et al, 1992, Schlesinger et al, 1996
E84K	II	conserved, E in TBE, K/R in others, attenuated by passage in <i>Ixodes ricinus</i> ticks, DII contains flavivirus cross reactive epitopes	TBE	Labuda et al, 1994
E85K	II	conserved, E in TBE, K/R in others, attenuation obtained as plaque variants in Vero cells, DII contains flavivirus cross reactive epitopes	JE	Wu et al, 1997
H104K	II	within highly conserved fusion peptide (aa 98-113), H in TBE, G in others	TBE	Rey et al, 1995
L107F	II	within highly conserved fusion peptide (aa 98-113), L in all flaviviruses, F in attenuated JE	TBE, JE, WN	Rey et al, 1995, Arroyo et al, 1999, 2004
T123K	II	DI- DII hinge, T in TBE, A in KFD	TBE	Holzmann et al, 1997
K126E	II	DI- DII hinge, K in TBE, E in D-2	DEN2	Bray, 98
K136E	II	DI- DII hinge, K in TBE and JE, E in D-2	JE	
N154L(Y)	I	glycosylation site, packed with conserved H 104, involved in fusion.	DEN2, DEN4, YF	Guirakhoo et al, 1993, Pletnev et al, 1993, Kawano et al, 1993, Jennings et al, 1994
K171E	I	external edge of DI, involved in fusion	TBE	Mandl, 1989, Holzmann, 1997
I173T		external edge of DI, involved in fusion	YF	Chambers and Nickells 2001
D181Y		DI- DII hinge	TBE	Holzmann et al, 1997
K204R		Lining Hydrophobic pocket, involve in	DEN1, DEN3	Guirakhoo et al, 2004

		fusion		
P272S	II	highly conserved, junction of one the of 2 alpha helices	JE	Cecilia et al, 1991
G308N	III	cell attachment, DKT in TBE, EGS in KFD, T-X in others, change to N produced glycosylation site in LI and reduced virulence. N-X-T/S glycosylation motif	LI	Jiang et al, 1993, Gao et al, 1994
S310K	III	putative cell attachment, change from E to G in JE reduced virulence	JE	Jiang et al, 1993, Gao et al, 1994, Wu et al, 1997
K311E	III	highly conserved, putative cell attachment	TBE, YF	Rey et al, 1995, Jennings, 1994
T333L	III	putative cell attachment	YF, LGT	Raynman et al, 1998
G334K	III	putative cell attachment	YF	Chambers and Nickells, 2001
S335K	III	putative cell attachment	JE	Wu et al, 1997
K336D	III	putative cell attachment	JE	Cecilia and Gould, 1991
P337D	III	putative cell attachment	JE	Cecilia and Gould, 1991
G368R	III	putative cell attachment	TBE, JE	Holzman et al 1997, Hasegawa et al 1992
Y384H	III	change to H attenuated TBE, putative cell attachment, -3 position to deleted RGD in TBE	TBE	Holzmann et al, 1990
V385R	III	conserved, -2 position to deleted RGD in TBE, putative cell attachment	D2	Hiramatsu et al, 1996, Lobigs, 1990
G386R	III	highly conserved, -1 position to deleted RGD in TBE, putative cell attachment	D2, MVE	Hiramatsu, 1996, Lobigs et al, 1990
E387R	III	conserved, +2 position to deleted RGD in TBE, putative cell attachment	D2, MVE	Hiramatsu, 1996, Lobigs et al, 1990
F403K	none	highly conserved, C-terminal region not included in crystal structure sE	D-2, D-4	Kawano et al, 1993, Bray et al, 1998
H438Y	None	highly conserved, C-terminal region not included in crystal structure sE	LGT	Campbell and Pletnev 2000
H496R	none	highly conserved, C-terminal region not included in crystal structure sE	TBE	Gritsun et al, 2001

References: Hasegawa et al., Virology 191(1):158-165; Schlesinger et al., J. Gen. Virol. 1996, 77 (Pt 6):1277-1285, 1996; Labuda et al., Virus Res. 31(3):305-315, 1994; Wu et al., Virus Res. 51(2):173-181, 1997; Holzmann et al., J. Gen. Virol. 78 (Pt 1):31-37, 1997; Bray et al., J. Virol. 72(2):1647-1651, 1998; Guirakhoo et al., Virology 194(1):219-223, 1993; Pletnev et al., J. Virol. 67(8):4956-4963, 1993; Kawano et al., J. Virol. 67(11):6567-6575, 1993; Jennings et al., J. Infect. Dis. 169(3):512-518, 1994; Mandl et al., J. Virol. 63(2):564-571, 1989; Chambers et al., J. Virol. 75(22):10912-10922, 2001; Cecilia et al., Virology 181(1):70-77, 1991; Jiang et al., J. Gen. Virol. 74 (Pt 5):931-935, 1993; Gao et al., J. Gen. Virol. 75 (Pt 3):609-614, 1994; Holzmann et al., J. Virol. 64(10):5156-5159, 1990; Hiramatsu et al., Virology 224(2):437-445, 1996; Lobigs et al., Virology 176(2):587-595, 1990; Campbell et al., Virology 269(1):225-237, 2000; Gritsun et al., J. Gen. Virol. 82(Pt 7):1667-1675, 2001.

Sequence Appendix 1

CV-TBEV Hypr or CV-LGT E5 with YFV/TBEV chimeric signal (p42, p59, and p43 constructs)

```

-----
YF17D partial signal
-----
TBEV partial signal
-----
Hypr or LGT E5 prM protein
-----
C protein YF17D
-----
R K R R S H D V L T V Q F L I L G M L G M T I A A T V R
A GGAACGCCG TTCCCATGAT GTTCGACTG TGCATTCCT AATTTGGG ATGCTGGGC TGACATCGC AGCTACGGTT CGC
T CCTTTGGCGC AAGGGTACTA CAAGACTGAC ACCTTAAGGA TTAACAACCG TAGGACCCGT ACTGTTAGCG TCGATGCCAA GCG
401

```

CV-TBEV Hypr with YFV/WNV chimeric signal (p45)

```

-----
C protein YF17D
-----
YF 17D partial signal
-----
WNV partial signal
-----
Hypr prM protein
-----
R K R R S H D V L T V Q F L I L G M L A C V G A A T V R
A GGAACGCCG TTCCCATGAT GTTCGACTG TGCATTCCT AATTTGGG ATGCTGGGC GTGTGGGAGC AGCTACCGTG CGA
T CCTTTGGCGC AAGGGTACTA CAAGACTGAC ACGTTAAGGA TTAACAACCG TAGGACCCGT CACAGCCTCG TCGATGGCAC GCT
401

```

RV-WNV/TBEV Hypr with TBEV signal (p39)

```

-----
TBEV signal
-----
WNV C protein
-----
Hypr prM protein
-----
Q K K R G G T D W M S W L L V I G M L G M T I A A T V R
CAAAGAAA CGGGGGGAA CAGACTGGAT GAGCTGGCTG CTCGTAATCG GCATGCTGGG CATGACAAATC GCAGCTACGG TTGCGC
GTTTTCTTT GCGCCCCCTT GTCTGACCTA CTCGACCGAC GAGCATTAGC CGTACGACCC GTACTGTTAG CGTCGATGCC AAGCG
201

```

RV-WNV/TBEV Hypr with WNV signal (p40)

```

-----
WNV signal
-----
WNV C protein
-----
Hypr prM protein
-----
Q K K R G G K T G I A V M I G M L A C V G A A T V R
CAAAGAAA CGGGGGGAA AGACAGGCAT AGCTGTGATG ATAGCATGCG TGGCTTGTTGT CGGAGCAGCT ACCGTGCGA
GTTTTCTTT GCGCCCCCTT TCTGTCCGTA TCGACACTAC TATCCGTAGC ACCGAACACA GCCTCGTCGA TGGCAGCCT
201

```

Sequence Appendix 2

CV-TBEV Hypr with YFV/TBEV chimeric signal (p42)

```

1  AGTAAATCCT GTGTGCTAAT TGAGGTGTCAT TGCTCTGCAA ATCGAGTGC TAGGCAATAA ACACATTGG ATTAATTTTA ATGCTTCGTT GAGCGATTAG
   5' UTR
TCAATTAGGA CACAGATTA ACTCCACGTA ACCAGACGTT TAGCTCAACG ATCCGTTAAT TGIGTAAACC TAATTAAAT TAGCAAGCAA CTCGCTAATC
-----
C protein
M S G R K A Q G K T L G V N M V R R G V R S L S N K I K
101 CAGAGAACTG ACCAGAACAT GTCTGTGCT AAAGCTCAGG GAAACACCT GGGCTCAAT ATGTACGAC GAGGAGTGC CTCCTTGTC AACAATAA
GTCTCTTGAC TGGTCTTGTA CAGACCAGCA TTTCGAGTCC CTTTGTGGA CCGCAGTTA TACCATGCTG CTCCTCAAGC GAGGAACAGT TTGTTTATT
C protein
Q K T K Q I G N R P G P S R G V Q G F I F F L F N I L T G K K I
201 AACAAAAAC AAACAAAT GGAACAGAC CTGGACCTTC AAGAGTGTT CAAGATTTA TCTTTTCTT TTGTTCAAC ATTTGACTG GAAAAAGAT
TTGTTTTTG TTTGTTTTAA CCTTGTCTG GACCTGGAAG TTCTCCAAA GTTCCTAAT AGAAAAAGAA AAACAAGTTG TAAACCTGAC CTTTTTCTA
C protein
T A H L K R L W K M L D P R Q G L A V L R K V K R V V A S L M R G
301 CACAGCCAC CTAAAGAGT TGTGGAAT GTTGGAACCA AGACAAGCTT TGGTGTCTT AAGGAAGTC AAGAGAGTGG TGGCCAGTTT GATGAGAGGA
GTGTCGGTG GATTTCTCCA ACACCTTTTA CGACCTGGT TCTGTCCGA ACCGACAAG TTCTCTCAG TTCTCTCACC ACCGGTCAA CTACTCTCT
YF17D partial signal
-----
TBEV partial signal
-----
C protein
L S S R K R R S H D V L T V Q F L I L G M L G M T I A A T V R K E R
401 TTGCTCTCAA GGAACGCG TTCCCATGAT GTTCTGACTG TGCATTCTT AATTTGGC ATGCTGGCA TGACAATGC AGCTACGTT CGCAAGGAAA
AACAGAGTT CCTTGGCGC AAGGTACTA CAAGACTGAC ACGTTAAGGA TTAAACCCG TAGCACCGT ACTGTTAGCG TCGATGCCAA GCGTTCTCTT
prM protein
D G S T V I R A E G K D A A T Q V R V E N G T C V I L A T D M G S
501 GAGACGGCAG TAGGTCTATA CGCGCGAAG GTAAGGATG CGTACCCAA GTGAGAGTGG AAAATGTGAC CTGCGTCATT CTGGCCACCG ACATGGGCTC
CTCTGCCGTC ATGCCAGTAT GCGGCGCTT CATTCCTACG CGGATGGGTT CACTCTCACC TTTTACCATG GACGACAGTA GACCGGTGGC TGTACCCGAG
prM protein
W C D D S L S Y E C V T I D Q G E E P V D V D C F C R N V D G V Y
601 TTGGTGTGAT GATAGCCTTT CTTATGAGTG CGTAACATA GATCAGGTG AGAACCTGT TGACTGTGAT TGCTTCTGCC GAAACGTGGA TGGGTGTAT
AACCACACTA CTATCGGAAA GAATACTCAC GCATTGGTAT CTAGTCCAC TCCTTGGACA CTGCAACTA ACAGAACGG CTTTGACCTT ACCCCACATA
prM protein
L E Y G R C G K Q E G S R T R R S V L I P S H A Q G E L T G R G H K
701 CTCGAATATG GACGGTGTGG TAAACAAGAA GGAAGCAGAA CCAGACGCTC AGTGCTTATA CCTCCACAG CTCAGGAGA GCTGACCGA CGGGACATA
GAGCTTATAC CTGCCACACC ATTTGTCTT CCTTCGCTT GGTCTGCGAG TCAGCAATAT GGGAGGTGC GAGTTCTCT CCACTGGCCT GCCCTGTAT

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prM protein

801 W L E G D S L R T H L T R V E G W V W K N R L L A L A M V T V V W
AATGTTGGA GGGCGACTCA CTCGAACAC ATTGACCG CGTCGAGGC TGGGTCTGA AAATCGGT GTTGCCCTC GCTATGGTA CAGTGGTTG
TTACCAACCT CCCGTGAGT GAGGCTTGT TAACTGGG GAGTCCCG ACCGACCT TTTAGCCGA CAACGGGAG CGATACCACT GTCAGCAAC

Hypr E protein

prM protein

901 L T L E S V V T R V A V L V V L L C L A P V Y A S R C T H L E N R
GCTCAGCTG GAGTCTGTG TTACTCGGT GGCAGTCTG GTGTCCTCC TGTCTTGC CCTGTCTAC CGCTCCAGT GTACTCATTT GGAACAACA
CGAGTGGAC CTCAGACACC AATGAGCGCA CCGTCACGAC CACCACGAG ACACAGAAG GGCACAGAT CGCAGGTCCA CATGAGTAAA CCTTTGTCT

Hypr E protein

1001 D F V T G T Q G T T R V T L V L E L G G C V T I T A E G K P S M D V
GATTTGTCA CCGGACCCCA GGGGACGACT CGGTAAACC TGGTCTGTA ACTGGTGT TCGTTACTA TTACCGTGA GGGCAACCC TCTATGGATG
CTAAACAGT GGCCGTGGT CCCCTGCTGA GCCATTGG ACCCAATGCT TGACCCACCA ACACAATGAT AATGGCGACT CCGTTTGG AGATACCTAC

Hypr E protein

1101 W L D A I Y Q E N P A Q T R E Y C L H A K L S D T K V A A R C P T
TGTGGCTGA TGCAATCTAT CAGGAGAATC CCGCACAAC CAGGGAATAT TGCCTTCACG CAAGCTGTC OGATACAAG GTCGGGCTA GGTGCCAAC
ACACGACCT ACCTTAGATA GTCTCTTAG GCGGTGTTG GTCCCTTATA ACAGGAGTGC GTTTGACAG GCTATGTTTC CAGCGCCGAT CCACGGGTTG

Hypr E protein

1201 M G P A T L A E E H Q G G T V C K R D Q S D R G W G N H C G L F G
AATGGACCG GCCACCTGG CCGAGGNAACA TCAGGAGGT ACAGTGTGCA AACGGGACCA GAGTGATAGA GGCTGGGTA ATCACTGGG CCTGTTGGC
TTACCTTGC CGGTGGGACC GCCTCTTGT AGTCCCTTCA GTTCACAGT TTGCTCTGT CTCACTATCT CCGACCCCAT TAGTGACGCC GGACAAGCCG

Hypr E protein

1301 K G S I V A C V K A A C E A K K K A T G H V Y D A N K I V Y T V K V
AAAGGAGTA TTGTCGCTG CGTCAAGCA GCTGTGAG CCAAAAGAA GGCTACTGG CACTCTATG ACCTCAACA GATGTTTAT ACAGTGAAG
TTTCTTCAT AACAGCGAAC GCATTCGCT CCGACACTCC GGTCTTCTT CCGATGACC GTGCAGATAC TGCGGTTGTT CTAGCAATA TGTCACTTTC

Hypr E protein

1401 E P H T G D Y V A A N E T H S G R K T A S F T V S S E K T I L T M
TGGACCAACA CACAGGGAT TACGTGGCG CCAACGAGAC TCATTCGGT CGCAAAACG CCAGCTTAC CGTGTCTAC GAAAGACCA TCCTCACTAT
ACCTTGTGT GTGTCCTTA ATGCACGCC GTTGTCTCTG AGTAAAGCCA GGTCTTGC GGTCAAGTGC GCACAGTAGG CTTTCTGCT AGGAGTGATA

Hypr E protein

1501 G E Y G D V S L L C R V A S G V D L A Q T V I L E L D K T V E H L
GGGAGATAT GGCAGCTTT CTGCTCTG CCGGTGGCT AGCGAGTGC ACCTGGCCA GACAGTCTC CTGGAACCTG ATAAACAGT TGAGCATCTG
CCCCCTCAT CCGCTGCAA GAGACGAGAC GGCCCAACA TCGCTCAGC TGGACCGGT CTGTCAGTAG GACCTTGACC TATTTGTCA ACTCGTAGAC

Hypr E protein

1601 P T A W Q V H R D W F N D L A L P W K H E G A R N W N N A E R L V E
CTACCGCTT GGCAGTGA CAGGATGG TTAAACACC TTGCTGACC ATGGAACAT GAAGAGCGA GAATCGAA TAATCGAG CGACTCGTAG
GGATGGGAA CCGTCCACGT GTCCCTAAC AAATGCTGG AACGGACCG TACCTTTGTA CTTCTCTGCT CTTGACCTT ATTACGCTC GCTGAGCATC

Hypr E protein

1701 F G A P H A V K M D V Y N L G D Q T G V L L K A L A G V P V A H I
AATCGGTGC CCCTCATGCC GTGAAGTGG AGCTCTACA TCTGGTGT CAGACCGGG TTTCTCTTAA AGCTCTGCT GCGTACCAAG TTGCCACAT
TTAAGCCAC GGGAGTACCG CACTTCTACC TGCAGATGT AGACCACTA GTCTGGCCG AAGAGGAT TCGAGAGCGA CCGCATGGT AACGGGTGA

Hypr E protein

1801 . E G T K Y H L K S G H V T C E V G L E K L K M K G L T Y T M C D K
CGAAGAACG AAGTACCACC TGAAGTCAGG CCATGTAAC TGGAGGTGG GCCTGGAGAA GTTGAATAAG AAAGGTCTTA CGTACACAAT GTGTGACAAG
GCTTCTCTGC TTCAAGGTGG ACTTCAGTCC GGTACATTTGA AGCTCCACC CGGACTCTT CAACTTTTAC TTCCAGAAAT GCATGTGTTA CACACTGTTC

Hypr E protein

1901 T K F T W K R A P T D S G H D T V V M E V T F S G T K P C R I P V R
ACCAAGTTCA CATGAAGAG GGGCCCAACA GATAGCGCC ACATGACTGT GGTGATGGAG GTGACCTTTT CTGGAACAAA ACCCTGCAGA ATACCGGTGC
TGGTTCAAGT GTACCTTCTC CCGGGGGTGT CTATGCGGG TGCTATGACA CCATACCTC CACTGSAAAA GACCTTGTTT TGGGACGTCT TATGGGCACG

Hypr E protein

2001 . A V A H G S P D V N V A M L I T P N P T I E N N G G G F I E M Q L
GGGCTGTAGC TCACGGATCT CCGCATGTCA ATGTGCTAT GCTGATATCA CCTAACCTTA CCATCGAGAA TAACGGTGGT GGTTTTATTG AGATGCAGCT
CCCGACATCG AGTGCCTAGA GGGCTACAGT TACAACGATA CGACTAATGT TATGTTGGAT GGTAGCTCTT ATTGCCACCA CCAAAATAAC TCTACGTGGA

Hypr E protein

2101 . P P G D N I I Y V G E L S Y Q W F Q K G S S I G R V F Q K T K K G
TCCGCCAGGC GATAACATCA TCTACGTGGG CGAATCTCT TACCACTGCT TACAGAGG GAGTTCAAT TCCAAAAAC GAAGAAGGA
AGGCGTCCG CTATTGTAGT AGATGCACCC GCTTGAGAGA ATGTACACCA AAGTCTTCC CTCGAAGTAA CCGGCCCAAG AGTTTITG CTCTCTCCCT

Hypr E protein

2201 I E R L T V I G E H A W D F G S A G G F L S S I G K A L H T V L G G
ATCGAACGAT TGACGGTTAT CGGCGAGCAC GATGGAAT TTGGTCCG AGGGGATTC CTGCTTCTTA TTGTAAGGC ACTGCATACC GTGCTGGGG
TAGCTTGCTA ACTGCCAATA GCGCTCGTG CGTACCTAA AACCAAGCG TCCCTTAAG GACAGAAGAT AACCAATCCG TGACGTATGG CACGACCCCC

Hypr E protein

2301 . A F N S I F G G V G F L P K L L L G V A L A W L G L N M R N P T M
CGCATTTCAA TTCTATTTTC GGGGGCGTGG GGTTCCTGCC TAAATCCTG CTGGAGTAG CCTGGCTG GTTGGACTG AATATGCGGA ATCCGACGAT
CGCGTAAGTT AAGATAAAAG CCGCGCACCC CCAAGGAGG ATTTGAGGAC GACCTCATC GGGACCGGAC CAACCTGAC TTATACGCT TAGGCTGCTA

Hypr E protein

2401 . S M S F L L A G V L V L A M T L G V G A D Q G C A I N F G K R E L
GTCCATGTCA TTCTCTTGG CCGGCTGCT TGTACTGGCC ATGACTGGC GGTGGCGC CGATCAAGGA TCGCCATCA ACTTTGGCAA GAGAGAGCTC
CAGTACAGT AAGGAGAAC GCGCGCACGA ACATGACCG TACTGTGACC CGCAACCGG GCTAGTTCTT ACGCGTAGT TGAACCGTT CTCTCTGAG

NS1 gene of YF17D

CV-TBEV Hypr with YFV/WNV chimeric signal (p45)

5' UTR

1 AGTAAATCCT GTGTGCTAAT TGAGGTGCAT TGGTCTGCAA ATCCAGTTGC TAGGCAATAA ACACATTTGG ATTAATTTTA ATCGTTCGTT GAGCGATTAG
TCATTAGGA CACACGATTA ACTCCACGTA ACCAGACGTT TAGCTCAACG ATCGTTATT TGTGTAACCC TAATTAAAT TAGCAAGCAA CTCGCTAATC

5' UTR

101 CAGAGAACTG ACCAGAACAT GTCTGTGCTG AAGTCTCAGG GAAAACCTT GGGGTCTAAT ATGGTAGGAC GAGGAGTTCG CTCCTTGTCA AACAAATAA
GTCTCTGAC TGGTCTTGTA CAGACAGCA TTTCAGTCC CTTTGTGGA CCGCAGTTA TACCATGCTG CTCCTCAAGC GAGGAACAGT TTGTTTATT

C protein YF17D

Q K T K Q I G N R P G P S R G V Q G F I F F F L F N I L T G K I

201 AACAAAAAC AAAACAAAT GGAACACAGC CTGGACCTTC AAGAGTGTT CAAGGATTTA TCTTTTCTT TTTGTTCAAC ATTTGACTG GAAAAAGAT
TTGTTTTTG TTTTGTAA CTTTGTCTG GACCTGGAAG TTCTCACAA GTTCTTAAT AGAAAAAGAA AAACAAGTG TAAACTGAC CTTTTTCTA
C protein YF17D

301 T A H L K R L W K M L D P R Q G L A V L R K V K R V V A S L M R G
CACAGCCAC CTAAGAGGT TGTGAAAT GTGGAGCCCA AGACAAGGT TGGTGTCTT AAGAAAGTC AAGAGAGTG TGGCCAGTTT GATGAGAGA
GTGTCGGGTG GATTTCTCA ACACCTTTTA CGACCTGGT TCTGTCCGA ACCACAAGA TTCTCTCAG TTCTCTCACC ACCGGTCAAA CTACTCTCT
C protein YF17D MNV partial signal

401 L S S R K R R S H D V L T V Q F L I L G M L A C V G A A T V R K E R
TTGTCTCAA GGAACGCC TTCCATGAT GTTCTAGT TCCATCTT TGAATCTT ATTTTGGC ATGCTGGCTT GTGTGGAGC AGTACCGTG CGAAAGAAG
AACAGGAGTT CTTTGGGC AAGGTACTA CAAGACTGAC AGTTAAGG TTAACCCG TAGACCCGA CACAGCTCG TCGATGGCAC GCTTTTCTT
Hypr prM protein

501 D G S T V I R A E G K D A A T Q V R V E N G T C V I L A T D M G S
CGCAGGAG CACGTGATA AGGCTGAGG GTAGGATGC GGCTAGCAG GTGAGAGTAG AGAATGGCAC TTGCGTAATA CTCGCACTG ATATGGGATC
CGTGCCTTC GTGGCACTAT TCCGACTCC CATTCCTACG CCGATGCGTC CACTCTCATC TCTTACCGTG AACGCAATTAT GAGCGCTGAC TATACCTAG
Hypr prM protein

601 W C D D S L S Y E C V T I D Q G E E P V D V D C F C R N V D G V Y
CTGTGTGAC GATAGCCTCA GTTATGAATG CTAACAATA GACAGGGCG AAGAACCCTT GGAGTTGAC TGTTCCTGTA GAAATGGA TGGCGTTTAT
GACCACACTG CTATCGGAGT CAATCTTAC GCATTGTTAT CTGTCCCGC TTCTGGACA CTTGCAACTG ACAAGACAT CTTTACACAT ACCGCAATA
Hypr prM protein

701 L E Y G R C G K Q E G S R T R R S V L I P S H A Q G E L T G R G H K
CTGGAGTAC GCCGTGTG AAAACAGAG GGTCAAGAA CTGAGATC TGTCTGAT CCAAGTCAG CCAAGGAGA GTTACCGGT AGAGGCCACA
GACCTCATGC CGCGACACC TTTTGTCTC CCGAGTCTT GAGCTTCTAG ACAGACTAA GGTTCAGTGC GGTTCCTCT CAACTGGCCA TCTCCGGTGT
Hypr prM protein

801 W L E G D S L R T H L T R V E G W V W K N R L L A L A M V T V W
AGTGGCTTGA AGGGACTCA TTGAGGACCC ACCTGACTAG GGTGAGGTT TGGTTTGA AGAATCGTT GCTCGGCTC GCTATGGTCA CCGTGTGTG
TCACGAACT TCCCTGAGT NACTCTGGG TGGACTGATC CCACCTCCA ACCCAACCT TCTTAGCCA CGAGCGGAG CGATACCAGT GGCAGCACAC
Hypr prM protein

901 L T L E S V V T R V A V L V V L L C L A P V Y A S R C T H L E N R
GCTGACACTG GAGAGTTCG TGAATCGGT TGTGTCTC GTTCTCTC CCAAGTGTAC CCGTCCAGT GTATCATTT GGAACACAGA
CGACTGTGAC CTTCAACAGC ACTGAGCCCA ACAGACAAAC CAACAGAGG AGACAAACCG GGTCCATAG CCGAGGTCCA CATGATGAAA CTTTTGTCT
Hypr E protein

1001 D F V T G T Q G T T R V T L V L E L G G C V T I T A E G K P S M D V
GATTTGTCA CCGCACCCA GGGGACACT CCGGTAAACC TGGTCTTGA ACTGGTGTG TGGTTACTA TTACCGTGA GGGCAACCC TCTATGGATG
CTAAACAGT GCGGTGGGT CCGCTGTGA GCGCATGG ACCAGAACT TGACCCACA ACAGAAATG AATGGCACT CCGTGTGG AGATACCTAC
Hypr E protein

1101 W L D A I Y Q E N P A Q T R E Y C L H A K L S D T K V A A R C P T
TGTGGCTGA TGAATCTAT CAGGAGATC CCGCAAAAC CAGGAATAT TGCCTTACG CAAAGTGTG CAGTACAAAG GTCGGGCTA GGTGCCAAC
ACACCGACCT ACCTTAGATA GTCTCTTAG GCGGTGTTG GTCCCTTATA ACAGAGTGC GTTTCGACAG GCTATGTTT CAGCGCGAT CCACGGGTTG
Hypr E protein

M G P A T L A E E H Q G G T V C K R D Q S D R G W G N H C G L F G

1201 AATGGGACCG GCCACCTGCG CGAGGAACA TCAGGAGGT ACAGTGTGA AACGGACCA GAGTGATAGA GGCTGGGTA ATCACTGGG CCTGTTCGGC
TTACCTTGGC CGGTGGACC GCCTCTTGT AGTCCCTCA TGTACAGT TTGCCCTGGT CTCACTAAT CCGACCCCAT TAGTGACGCC GGACAAGCCG

K G S I V A C V K A A C E A K K K A T G H V Y D A N K I V Y T V K V
1301 AAAGGAAGTA TTGTGCTTG CGTCAAGCA GCTGTGAGG CCAAAAGAA GGCTACTGG CACGTCTATG ACGCAACAA GATCGTTTAT ACAGTGAAG
TTTCCTTCAT AACAGGNAAC GCAGTTCCGT CGGACACTCC GGTTTTCTT CCGATGACCC GTGCAGATAC TGCCTTGT CTAGCAATA TGTCACTTTC

Hypr E protein

E P H T G D Y V A A N E T H S G R K T A S F T V S S E K T I L T M
1401 TGGAAACACA CACAGGGAT TACGTGGCG CCAACGAGC TCATTCGGT CGCAACGG CCAGTTTAC CGTGTATCC GAAAGACCA TCCTCACTAT
ACCTTGGTGT GTGTCCCTTA ATGCACGCC GGTGTCTCTG AGTAAGCCA CGTTTGTCC GGTCAAGTG GCACAGTAGG CTTTCTGTGT AGGAGTGATA

Hypr E protein

G E Y G D V S L L C R V A S G V D L A Q T V I L E L D K T V E H L
1501 GGGGAGTAT GCGAGCTTT CTCTGCTCTG CCGGTGCTG ACGGAGTGC ACCTGGCCCA GACATCATC CTGGAACCTGG ATAAACAGT TGAGCATCTG
CCCCCTCATA CCGCTGCAAA GAGAGAGAC GGGCCACCA TGCCTCAGC TGGACCGGT CTGTCACTAG GACCTTGACC TATTTGTCTA ACTCGTAGAC

Hypr E protein

P T A W Q V H R D W F N D L A L P W K H E G A R N W N N A E R L V E
1601 CCTACGCTT GGCAGTGCA CAGGATGG TTAAACGACC TTGCCCTGCC ATGGAACAT GAAGAGCGA GAACTGGAA TAATGCAGAG CGACTCGTAG
GGATGGGAA CCGTCCAGT GTCCCTAACC AAATTGCTGG AACGGAGCG TACCTTTGTA CTTCCTGCT CTTTGACCTT ATTACGTTCT GCTGAGCATC

Hypr E protein

F G A P H A V K M D V Y N L G G D Q T G V L L K A L A G V P V A H I
1701 AATTCGCTG CCTCATGCC GTGAGTGG AGCTCTACA TCTGGTGTAT CAGCGGGG TTCTCTTAA AGCTCTGCT GCGTACAGT TGCCCCACAT
TTAAGCCAG GGGAGTACG CACTTCTACC TGCAGATGT AGACCCACTA GTCTGCGCG AGAGGAAT TCGAGAGCGA CCGCATGGTC AACGGGTGA

Hypr E protein

E G T K Y H L K S G H V T C E V G L E K L K M K G L T Y T M C D K
1801 CGAAGAACG AAGTACCACC TGAAGTCAG CCATGTAAT TCGAGTGG GCCTGAGAA GTTGAATAAG AAGGTCTTA CGTACACAT GTGTGACAAG
GCTTCCTGC TTATGGTGG ACTTCAGTCC GGTACATGA AGCTCCACC CCGACTCTT CAACCTTTAC TTTCAGAAAT GCATGTCTTA CACACTGTTC

Hypr E protein

T K F T W K R A P T D S G H D T V V M E V T F S G T K P C R I P V R
1901 ACCAAGTCA CAGGAAGAG GGGCCCAACA GATAGCGCC AGATATCTGT GGTGATGGAG GTGACCTTTT CTGGAACAAA ACCCTGCAGA ATACCGTGC
TGGTTCAAGT GTACCTTCTC CCGGGGGTGT CTATGCGCG GTCTATGACA CCACTACCTC CACTGGAATA GACCTTGTTC TGGGACGTCT TATGGGACG

Hypr E protein

A V A H G S P D V N V A M L I T P N P T I E N N G G G F I E M Q L
2001 GGGCTGTAGC TCACGGATCT CCGGATGCA ATGTTGCTAT GCTGATACA CCTAACCTTA CCATCGAGAA TACGGTGGT GGTTTTATG AGATGAGCT
CCGACATCG AGTGCCCTAGA GGGCTACAGT TACAACGATA CGACTAATGT GGATGGAT GGTAGTCTT ATTGCCACCA CCAAAATAAC TCTACCTGA

Hypr E protein

P P G D N I I Y V G E L S Y Q W F Q K G S S I G R V F Q K T K K G
2101 TCCGCGAGC GATAACATCA TCTACGTGG CGAATCTCT TACCAGTGT TACAGAAAG GAGTTCAAT GGGCGGTCT TCCAAAAC GAAGAAGGA
AGGCGTCCG CTATGTAGT AGATGCACC GCTTGAGAGA ATGGTACCA AAGTCTTCC CTCAAGTTAA CCGGCCCAAG AGGTTTTTG CTCTCTCCCT

Hypr E protein

I E R L T V I G E H A W D F G S A G G F L S S I G K A L H T V L G G
2201 ATCGAACGAT TGACGGTAT CCGGAGCAC GGTGCGAT TTGGTCCG AGGGGATTC CTCTCTTA TTGGTAAGC ACTGCATACC GTGCTGGGG
TAGCTTGCTA ACTGCCATA GCGCTCTG CGTACCTTAA AACCAAGCG TCCCCTAAG GACAGAAGT AACCATCCG TGACGTATGG CACGACCCC

Hypr E protein

2301 . A F N S I F G G V G F L P K L L L G V A L A W L G L N M R N P T M .
 GCGCATTCAA TTCTATTTC GGGGCGTGG GGTTCCTGCC TAACTCCTG CTGGAGTAG CCTGGCCTG GTTGGACTG AATATGCGA ATCCGACGAT
 CGCGTAAGTT AAGATAAAG CCCCACCA CCAAGCAGG ATTTGAGGAC GACCTCATC GGGACCGGAC CAACCTGAC TTATACGCT TAGGCTGCTA

Hypr E protein

NS1 gene of YF17D

2401 . S M S F L L A G V L V L A M T L G V G A D Q G C A I N F G K R E L .
 GTCCATGTCA TTCTCTTGG CCGCGTGTCT TGTACTGGCC ATGACACTGG CGGTGGCGC CGATCAAGGA TCGCCCATCA ACTTTGGCAA GAGAGAGCTC
 CAGGTACAGT AAGGAGAACC GCGCGCAGGA ACATGACCGG TACTGTACC CGCAACCGCG GCTAGTTCCT ACGCGGTAGT TGAACCGTT CTCTCTCGAG

CV-LGTV E5 with YFV/TBEV chimeric signal (p43)

5' UTR

1 AGTAAATCCT GTGTGCTAAT TGAGGTGCAT TGGTCTGCAA ATCGAGTTGC TAGGCAATAA ACACATTTGG ATTAATTTTA ATCGTTCTGT GAGCGATTAG
 TCATTTAGGA CACACGATTA ACTCCACGTA ACCAGAGGTT TAGTCAACG ATCCGTTATT TGTGTAAACC TAATTTAAAT TAGCAAGCAA CTCGCTAATC
 5' UTR

C protein YF17D

101 M S G R K A Q G K T L G V N M V R R G V R S L S N K I K .
 CAGAGAACTG ACCAGAACAT GTCTGTGCT AAAGCTCAGG GAAAACCCCT GGGGTCAAT ATGGTACGAC GAGGAGTTG CTCCTGTGCA ACAAATAA
 GTCTCTTGAC TGGTCTTGTA CAGACCAGCA TTTCGAGTCC CTTTGTGGA CCGCAGTTA TACCATGCTG CTCCTCAAGC GAGGAACAGT TTGTTTATT
 C protein YF17D

201 . Q K T K Q I G N R P G P S R G V Q G F I F F L F N I L T G K K I .
 AAAAAAAC AAAAAATT GGAACAGAC CTGGACCTTC AAGAGTGT TCAAGATTTA TCTTTTCTT TTGTGTCAC ATTTGACTG GAAAAAGAT
 TTGTTTTTGT TTTGTTTAA CTTTGTCTG GACCTGGAAG TTCTCCACAA GTTCTTAAT AGAAAAAGAA AACAAGTTG TAAACTGAC CTTTTTCTA
 C protein YF17D

301 . T A H L K R L W K M L D P R Q G L A V L R K V K R V V A S L M R G .
 CACAGCCAC CTAAGAGGT TGTGAAAT GCTGACCCA AGACAAGGT TGGTGTCTT AAGGAAGTC AAGAGAGTGG TGGCCAGTTT GATGAGAGGA
 GTGTGGGTG GATTCTCCA ACACCTTTA CGACCTGGGT TCTGTCCGA ACCGACAGA TTCTTTTCTG TTCTCTCACC ACCGTCAAA CTACTCTCCT
 C protein YF17D

TBEV partial signal

YF 17D partial signal

prM protein Langat E5

401 L S S R K R R S H D V L T V Q F L I L G M L G M T I A A T V R R E R .
 TTGTCTCAA GGAACGCG TTCCCATGAT GTTCTGACTG TGCATTCTT AATTGCGC ATGCTGGGA TGACGATCGC AGTACTGTG CGAAGGGAGA
 AACAGGAGTT CCTTGGCGC AAGGGTACTA CAAGACTGAC ACGTAAAGGA TTAACACCGG TACGACCCCT ACTGCTAGCG TCGATGACAC GCTTCCCTCT
 prM protein Langat E5

501 . D G S M V I R A E G R D A A T Q V R V E N G T C V I L A T D M G S .
 GAGAGGCTC TATGGTGATC AGAGCGAAG GTAGGACGC TGGACCCAG GTGAGGGTCG AAAATGGCAC CTGTGTATT CTGGGACCG ACATGGGCTC
 CTCTGCCGAG ATACCACTAG TCTCGGCTC CATCCCTGC ACCTGGGTC CACTCCGAC TTTTACCGTG GACACAATAA GACCGCTGC GTTACCCGAG
 prM protein Langat E5

601 . W C D D S L A Y E C V T I D Q G E E P V D V D C F C R G V E K V T .
 CTGGTGTGAT GATTCTCTG CTTATGAATG TGTACTATT GATCAGGGTG AGAGCCTGT GGACGTGGAC TGTTTCTGTA GAGCGTCGA GAAAGTGACC
 GACCACACTA CTAAGAGACC GAATACTTAC ACAATGATAA CTAGTCCAC TTCTGGACA CCTGCACCTG ACAAGACAT CTCGCGAGCT CTTTCACTGG

701	prM protein Langat E5																			
	L	E	Y	G	R	C	G	R	R	E	G	S	R	S	R	S	V	L	I	P
801	prM protein Langat E5																			
	W	L	E	G	E	A	V	K	A	H	L	T	R	V	E	G	W	V	K	N
901	E protein Langat E5																			
	L	M	V	D	G	L	L	P	R	I	L	I	V	V	V	A	L	A	L	A
1001	E protein Langat E5																			
	D	F	V	T	G	V	Q	G	T	T	R	L	T	L	V	L	E	L	G	G
1101	E protein Langat E5																			
	W	L	D	S	I	Y	Q	E	S	P	A	Q	T	R	E	Y	C	L	H	A
1201	E protein Langat E5																			
	M	G	P	A	T	L	P	E	E	H	Q	S	G	T	V	C	K	R	D	Q
1301	E protein Langat E5																			
	K	G	S	I	V	T	C	V	K	V	T	C	E	D	K	K	A	T	G	H
1401	E protein Langat E5																			
	E	P	H	T	G	E	F	V	A	A	N	E	T	H	S	G	R	K	S	A
1501	E protein Langat E5																			
	G	D	Y	G	D	V	S	L	L	C	R	V	A	S	G	V	D	L	A	Q
1601	E protein Langat E5																			
	P	T	A	W	Q	V	H	R	D	W	F	N	D	L	A	L	P	W	K	H

1701 . F G T P H A V K M D V F N L G D Q T G V L L K S L A G V P V A S I .
 AATTTGGAAC CCACACGCC GTAAAGATGG ACCTTTTCAA TCTTGGTGAC CACACAGGG TGCTCTGAA ATCACTGGCG GCGTGGCTG TAGCCAGCAT
 TTAACCTTG GGGTGTGCG CATTCTACC TCGAAAGATG AGAACCACTG GTCGTGCC ACAGGACTT TAGTGACCG CCGACGGAC ATCGGTGCTA
 E protein Langat E5

1801 . E G T K Y H L K S G H V T C E V G L E K L K M K G L T Y T V C D K
 CGAGGGACA AAGTATCAC TGAAGTCTGG GCATTAACC TCGAAGTGG GCTTGGAAA GCTGAAGATG AAAGGACTTA CGTACACTGT TTGTGATTAAG
 GCTCCCGTGT TTCATAGTGG ACTTCAGACC CGTACATCG AGCTTCACC CCGACTTTT CGACTTCTAC TTTCCTGAAT GCATGTGACA AACACTATTC
 E protein Langat E5

1901 T K F T W K R A P T D S G H D T V V M E V G F S G T R P C R I P V R .
 ACCAAGTTA CATGGAAGC AGCCCAACG GATTCGGCC ATGATACCTT CGTGATGGAG GTTGGTTTCT CCGGACCCAG ACCATGTAGA ATACCAGTGA
 TGGTTCAAAT GTACCTTCG TCGGGGTGC CTAAGCCCG TACTATGGA GCATACCTC CAACCAAGA GCGCGTGGT TGTATCATCT TATGTCCTCT
 E protein Langat E5

2001 . A V A H G V P E V N V A M L I T P N P T M E N N G G G F I E M Q L .
 GAGTGTCC CCACGGTGA CCGAGGTAA ACGTGGCCAT GCTGATTACA CCGAATCCA CTATGGAGAA CAATGCGGA GGGTTCATCG AATGCAGCT
 CTCGACAGG GGTGCCACAT GGGCTCCATT TGCACCGTA CGACTAATGT GGTTAGGGT GATACCTCTT GTTACCGCCT CCCAAGTAGC TTACGTCGA
 E protein Langat E5

2101 . P P G D N I I Y V G D L D H Q W F Q K G S S I G R V L Q K T R K G
 GCGCCTGA GACACATCA TTTATGCG CGACTCGAT CATCAATGT TCCAGAAGG GTCTTCCATC GCGCGCTCC TTCAGAAGAC ACGAAGAAGC
 CCGGGACCT CTGTGTAGT AAATACAGC GCTGAGCTA GTAGTTACA AGTCTTTC CAGAGGTAG CCGGCGCAGG AAGTCTTCTG TGCCTTTCCG
 E protein Langat E5

2201 I E R L T V L G E H A W D F G S V G G V M T S I G R A M H T V L G G .
 ATTGAAGAC TTACAGTCTT GGGCGACAT GCCTGGACT TCGGTCTAGT TCGCGGGTA ATGACAGCA TAGGCAGAGC TATGCACACC GTTCTCGGTG
 TAACTTTCTG AATGTCAGGA CCGCTTGTA CCGACCTGTA AGCCAGTCA ACCGCCCAT TACTGTCTGT ATCCGTCTCG ATCCGTGTGG CAAGAGCCAC
 E protein Langat E5

2301 . A F N T L L G G V G F L P K I L L G V A M A W L G L N M R N P T L .
 GGGCAATTAA TACTCTGTG GGTGGGTGG GTTCTTCTC GAAATCTCTG CTGGTCTCG CAATGSCCTG GCTTGGACTG AATATGCGCA ATCTACACT
 CCGTAAAT ATGAGACAAC CCACCGCAC CAAAGAAGG CTTTATAGAC GAGCCACAGC GTTACCGGAC CGAACCTGAC TTATACGCGT TAGGATGTGA
 E protein Langat E5

2401 . S M G F L L S G G L V L A M T L G V G A D Q G C A I N F G K R E L
 GAGTATGGG TTTCTTCTGT CAGGAGCCT GGTCTGGA ATGACTCTGG GAGTGGCGC CGATCAAGGA TGCGCATCA ACTTTGGCAA GAGAGAGCTC
 CTCATACCCC AAAGAAGACA GTCTCTCCGA CCAGGACCGT TACTGAGACC CTCACCGCG GCTAGTTCCT ACGCCGTAGT TGAACCCGTT CTCTCTCGAG
 NS1 gene of YF17D

CV-TBEV Hypr with YFV/TBEV chimeric signal and dC2 deletion in C protein (p59)

5' UTR

1 AGTAAATCCT GTGTGCTAAT TGAGTGCAT TGGTCTGCAA ATCGAGTTC TAGGCAATAA ACACATTGG ATTAATTTTA ATCGTTCTGTT GAGCGATTAG
 TCATTTAGGA CACACGATTA ACTCCAGTA ACCAGACGTT TAGCTCAACG ATCCGTTATT TGTGTAACC TAAITAAAT TAGCAAGCAA CTCGCTAATC
 5' UTR

C protein

101 M S G R K A Q G K T L G V N M V R R G V R S L S N K I K
CAGAGACTG ACCAGACAT GTCTGGTCTT AAAGCTCAGG GAAACACCTT GGGCTCAAT ATGTACGAC GAGGAGTTCG CTCCTTGTCA AACAAATAA
GTCTCTTGAC TGGTCTTGTA CAGACCAGCA TTCTGGATCC CTTTGGGA CCCGAGTTA TACCATGCTG CTCTCAAGC GAGGAACAGT TTGTTTATT
dc2 deletion (PSR)

C protein

201 Q K T K Q I G N R P G G V Q G F I F F L F N I L T G K K I T A H
AACAAAAAC AAAACAAAT GGAACAGAC CTGGAGTGT TCAAGATT ATCTTTTCT TTGTGTTCA CATTTGACT GGAATAAGA TCACGCCCA
TTGTTTTTG TTTGTTTAA CCTTGTCTG GACCTCCACA AGTTCCTAAA TAGAAAAAGA AAAACAAGT GTAAAACTGA CCTTTTCT AGTGTGGGT
C protein

301 L K R L W K M L D P R Q G L A V L R K V K R V V A S L M R G L S S
CCTAAGAGG TTGTGAAA TGTGGACCC AAGACAGGC TTGGCTGTTT TAAGAAAGT CAAGAGAGT GTGGCCAGTT TGATGAGG ATTGTCTCA
GGATTCTCC AACACCTTTT ACGACCTGG TTCTGTTCG AACCGACAAG ATTCCTTTCA GTTCTCTCAC CACCGGTCAA ACTACTCTCC TAACAGGAGT
YF17D partial signal

TBEV partial signal

C protein

401 R K R S H D V L T V Q F L I L G M L G M T I A A T V R K E R D G S
AGGAAGGCC GTTCCATGA TGTCTGACT GTCAATCC TAATTTGG CATGCTGGC ATGCAATCG CAGTACGGT TCGCAAGAA AGAGACGGCA
TCCTTGGCG CAGGGTACT ACAAGACTGA CAGTTAAG ATTAACCC GTACACCCG TACTGTAGC GTGATGCCA AGGTTCTCTT TCTGTGCCG
Hypr prM protein

501 T V I R A E G K D A A T Q V R V E N G T C V I L A T D M G S W C D
GTACGGTCAAT ACGGCGGAA GGTAAAGATG CCGTACCCA AGTAGAGTG GAAATGTTA CTGCGTCAT TGTGGCCACC GACATGGCT CTTGGTGTGA
CATGCCAGTA TGCGGCCCTT CCATTCTTAC GCGATGGT TCACCTCAC CTTTACCAT GGAGCAGTA AGACCGGTG CTGTACCGA GAACCACT
Hypr prM protein

601 D S L S Y E C V T I D Q G E E P V D V D C F C R N V D G V Y L E Y
TGATAGCCTT TCTTATGAGT GCGTAACCAT AGATCAAGT GAGAACCTG TTGAGTTGA TTGCTTCTG CGAAACCTGG ATGGGTGTA TCTCGAATAT
ACTATCGGAA AGAATACTCA CGCATTTGTA TCTAGTTCCA CTCCTTGGAC AACTGCACT AACGAAGACG GCTTTGCACC TACCCACAT AGAGCTTATA
Hypr prM protein

701 G R C G K Q E G S R T R R S V L I P S H A Q G E L T G R G H K W L E
GGACGGTGTG GTAAACAAGA AGGAAGCAGA ACCAGAGCT CAGTCTTAT ACCCTCCAC GCTCAAGAG AGCTGACCGG ACGGGACAT AAATGGTTGG
CCTGCCACAC CATTTGTTCT TCCTTCGTCT TGGTCTGGA GTCAGAAAT TGGAGGGTG CGAGTTCTCT TCGACTGGCC TGCCCTGTA TTACCAACC
Hypr prM protein

801 G D S L R T H L T R V E G W V W K N R L L A L A M V T V V W L T L
AGGGGACTC ACTCCGAACA CATTTGACCC CGTCCAGG CTGGTCTGG AAAATCGG TGTGGCCT CGTATGGT ACAGTGTGTT GGTCACGCT
TCCCGCTGAG TGAGGCTTGT GTAAACTGG GCGAGTCCC GACCCAGACC TTTTATGCG ACAACCGGA CGATACCAAC TGTCAGCAA CCGAGTGGCA
Hypr E protein

Hypr prM protein

901 E S V V T R V A V L V V L L C L A P V Y A S R C T H L E N R D F V
GGAGTCTGT GTTACTCGG TGGCAGTGT GGTGGTCTC CTCTGTCTT CCCTGTCTA CCGTCCAGG TGTACTCAT TGGAAACAG AGATTTGTC
CCTCAGACAC CAATGAGC CCGTTCAGA CCACACAG GAGACAGAC GGGACAGAT GCGCAGTCC ACATGAGTAA ACCTTTGTC TCTAAACAG
Hypr E protein

T G T Q G T T R V T L V L E L G G C V T I T A E G K P S M D V W L D

1001	ACCGCACCC AGGGGACGAC TCGGGTAACC CTGGTGTCTG AACTGGGTGG TTGCGTTACT ATTACCGGTG AGGGCAAACC CTCTATGGAT GTGTGGCTGG TGGCGTGGG TCCCGTCTG AGCCCAITGG GACCACGAAC TTGACCACC AACGCAATGA TAATGGCGAC TCCCGTTTGG GAGATACCTA CACACCGACC ----- Hypr E protein
1101	· A I Y Q E N P A Q T R E Y C L H A K L S D T K V A A R C P T M G P · ATGCAATCTA TCAGGAGAAT CCGCACAAA CCAGGGAATA TTGCTTTCAC GCAAGCTGT CCGATACAAA GGTGCGGCT AGGTGCCCAA CAATGGGACC TACGTTAGAT AGTCCTCTTA GGGCGTGTGT GTTCCTTTAT AACGGAATG CGTTTGACA GGCTATGTT CCAGCGCOGA TCCACGGGTT GTTACCCTGG ----- Hypr E protein
1201	· A T L A E E H Q G G T V C K R D Q S D R G W G N H C G L F G K G S · GGCCACCTG GCGGAGAAC ATCAGGAGG TACAGTGTG AACGAGACC AGAGTGATAG AGGTGGGT AATCACTGG GCTGTTCGG CAAAGGAAGT CCGGTGGGAC CGCTCTCTTG TAGTCCCTCC ATGTCACAG TTTCCTCTGG TCTCACTATC TCCGACCCCA TTAGTGACGC CGGACAAGCC GTTTCCTTCA ----- Hypr E protein
1301	· I V A C V K A A C E A K K A T G H V Y D A N K I V Y T V K V E P H · ATTGTGCTT GGTCAAGC AGCTGTGAG GCCAAAAGA AGGTACTGG GCAGTCTAT GACGCCAACA AGATCGTTTA TACAGTGAAA GTGGAACCAAC TAACAGCGAA CGCAGTTCCG TCGGACACTC CGGTTTTCT TCCGATGACC CGTGCGAAGT GGCACAGTAG GCTTTCTGG TAGGAGTGAT ACCCCCTCAT ----- Hypr E protein
1401	· T G D Y V A A N E T H S G R K T A S F T V S S E K T I L T M G E Y · ACACAGGGA TTACGTGGG GCCAACGAGA CTCATTCCGG TCGCAAACG GCCAGTTCA CCGTGTATC CGAAAGACC ATCTCACTA TGGGGGAGTA TGTGTCCCT AATGACCGC CGGTTGCTCT GAGTAAGGCC ACGTTTGC CGTGCGAAGT GGCACAGTAG GCTTTCTGG TAGGAGTGAT ACCCCCTCAT ----- Hypr E protein
1501	· G D V S L L C R V A S G V D L A Q T V I L E L D K T V E H L P T A · TGGCAGCTT TCTGTCTCT GCGGGTGC TAGCGGAGT GACCTGCC AGACAGTCT CCTGGAACGT GATAAAACAG TTGAGCATCT GCCTACCGT ACCGTGCAA AGAGAGAGA CGGCCACCG ATGCGCTCAG CTGGACCGG TCTGTGAGTA GGACCTTGAC CTATTTTGT AACTCGTAGA CGGATGGCGA ----- Hypr E protein
1601	· W Q V H R D W F N D L A L P W K H E G A R N W N N A E R L V E F G A · TGGCAGTGC ACAGGATTG GTTAAACGAC CTGCGCTGC CATGGAACA TGAAGAGCG AGAACTGGA ATATGAGA GCGACTCGTA GAATTCGGT ACCGTCCACG TGTCCTAAC CAAATTGCTG GAACGGGAC GTACCTTGT ACTTCTGC TCTTTCACCT TATTACGCT CGCTGAGCAT CTTAAGCCAC ----- Hypr E protein
1701	· P H A V K M D V Y N L G D Q T G V L L K A L A G V P V A H I E G T · CCCCTCATG CGTGAAGATG GACGTCTACA ATCTGGGTGA TCAGACCGG GTTCTCTTA AGCTCTCGC TGGCGTACCA GTTGCCACA TCGAAGAAC GGGAGTAGC GCATTCTAC CTGCAGATGT TAGACCCACT AGTCTGCGC CAAGAGGAAT TTGAGAGCG ACCGCAATGT CAACGGGTGT AGCTTCCTTG ----- Hypr E protein
1801	· K Y H L K S G H V T C E V G L E K L K M K G L T Y T M C D K T K F · GAAGTACCAC CTGAAGTCAG GCCATGTAAC TTGCGAGTG GGCCTGAGA AGTTGAAAAT GAAAGTCTT ACGTACACAA TGTGTGACAA GACCAAGTTC CTTCATGGTG GACTTCAGTC CGGTACATG AAGCTCCAC CGGACCTCT TCACTTTTA CTTTCCAGAA TGCATGTGTT ACACACTGTT CTGTTTCAAG ----- Hypr E protein
1901	· T W K R A P T D S G H D T V V M E V T F S G T K P C R I P V R A V A · ACATGAAGA GGGCCCCAC AGATAGCGC CACGATCTG TGGTATGGA GGTGACCTTT TCTGGAACAA AACCTCGAG AATACCGTG CGGGCTGTAG TGTACTTCT CCCGGGGTG TCTATGCGG GTGCTATGAC ACCACTACCT CCAGTGGAA AGACCTTGT TTGGGACGTC TTATGGCAC GCGGACATC ----- Hypr E protein
2001	· H G S P D V N V A M L I T P N P T I E N N G G G F I E M Q L P P G · CTCAGGATC TCCGATGTC AATGTTGCTA TGCTGATAC ACCTAACCTT ACCATCGAGA ATACGGTGG TGGTTTTATT GAGATGACG TTCCGCCAGG GAGTGCCTAG AGGCTACAG TTACAACGAT ACCACTAATG TGGATTGGA TGAGTCTCT TATTGCCACC ACCAATAA CTCTACGTCG AAGCGGTCC ----- Hypr E protein

2101 . D N I I Y V G E L S Y Q W F Q K G S S I G R V F Q K T K K G I E R
CGATAACATC ATCTAGTGG GCGAACTCTC TTACAGATGG TTTCAGAAAG GGAGTTCAAT TGGCGGGTC TTCCAAAAA CGAAGAAGG AATCGAACGA
GCTATTGTAG TAGATGCACC CGCTTGAGAG AATGGTCACC AAGTCTTTC CCTCAAGTTA ACCCGCCAG AAGTTTTTT GCTTCTTCCC TTAGCTTGCT

Hypr E protein

2201 L T V I G E H A W D F G S A G G F L S S I G K A L H T V L G G A F N
TTGACGGTTA TCGGCGAGCA CGCATGGGAT TTGGGTTCCG CAGGGGATTT CCTGTCTTCT ATTGTAAG CACTGCATAC CGTGTGGG GCGGCATTCA
AACTGCCAAT AGCCGCTCGT GCGTACCCTA AAACCAAGGC GTCCCCCTAA GGACAGAAGA TAACCATTC GTGACGTATG GCACGACCCC CGCGTAAAGT

Hypr E protein

2301 . S I F G G V G F L P K L L L G V A L A W L G L N M R N P T M S M S
ATTCTATTTT CGGGGCGTG GGGTTCCTGC CTAACCTCT GCTGGAGTA GCCCTGSCCT GGTTGGGACT GAATATGCGG AATCCGACGA TGTCATGTTC
TAAGATAAAA GCCCCGAC CCCAAGGACG GATTGAGGA CGACCTCAT CCGGACCGGA CCAACCTGA CTTATACGCC TTAGGCTGCT ACAGGTACAG

Hypr E protein

NS1 gene of YF17D

2401 . F L L A G V L V L A M T L G V G A D Q G C A I N F G K R E L
ATTCTCTTTC GCGGCGTGC TTGTACTGCG CATGACACTG GCGGTGGCG CCGATCAAGG ATGCGCCATC AACTTTGGCA AGAGAGAGCT C
TAAGGAGAAC CGGCGGACG AACATGACCG GTACTGTGAC CGCAACCGC GGCTAGTTC TACGCGGTAG TTGAACCCGT TCTCTCTCGA G

Sequence Appendix 3

PIV-WN/TBEV Hypr with TBEV signal (p39)

5' UTR

deleted C

1 AGTAGTTGCG CTGTGTGAGC TGACAAACTT AGTAGTGTTC GTGAGGATTA ACAACAATTA ACACAGTGGC AGCTGTTTCT TAGCACGAAG ATCTCGATGT
TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCCTAAT TGTTGTTAAT TGCTCACGC TCGACAAAGA ATCGTGTCTC TAGAGCTACA

WNV deleted C protein

101 . K K P G G P G K S R A V Y L L K R G M P R V L S L I G L K R S S K
CTAAGAAACC AGGAGGCCC GGCAAGAGCC GGGTGTCTA TTGTCTAAA CGCGGAATGC CCGCGTGT GTCTTGATT GGACTTAAGC GGAGCTCCAA
GATTCCTTGG TCCTCCCGG CGGTCTCGG CCGACAGAT AAACGATTTT GCGCTTACG GGGCGCACAA CAGGAACCTAA CCGTAATTCG CCTCGAGGTT

TBEV signal

deleted C

prM Hypr

201 . Q K K R G G T D W M S W L L V I G M L G M T I A A T V R K E R D G
ACAAAAGAAA CGGGGGGAA CAGACTGGAT GAGCTGGCTG CTGTAATCG GCATGCTGG CATGACAATC GCAGTACGG TTGCGAAGGA AAGAGACGGC
TGTTTCTTTT GCCCCCCTT GTCTGACCTA CTGACCGAC GAGCATTAGC CGTACGACC GTACTGTTAG CGTGTATGCC AAGCTTCTT TCTCTGCG

prM Hypr

301 S T V I R A E G K D A A T Q V R V E N G T C V I L A T D M G S W C D
AGTACGGTCA TACGCGCGGA AGTAAGGAT GCGGTACCC AAGTGAGAGT GGAAATGTT ACCTGCGTCA TTCTGGCCAC CGACATGGGC TCTTGCTGTG

TCATGCCAGT ATGCGCGCCT TCCATTCTTA CGGCGATGGG TTCACTCTCA CTTTTTACCA TGGACGAGT AAGACCGGT GCTGTACCCG AGRACCACAC
 prM Hypr

401 D S L S Y E C V T I D Q G E E P V D V D C F C R N V D G V Y L E Y
 ATGATAGCCT TTCTTATGAG TGCCTAACCA TAGATCAAGG TGAGGAACCT GTTGACCTTG ATTGCTTCTG CCGAAGCTG GATGGGGTGT ATCTCGAATA
 TACTATCGGA AAGAACTACTC AGCAATTGGT ATCTAGTTCC ACTCCTTGGA CAACTGCAAC TAACGAAGAC GGCCTTGCAC CTACCCCACTA TAGAGCTTAT
 prM Hypr

501 G R C G K Q E G S R T R R S V L I P S H A Q G E L T G R G H K W L
 TGACGGTGT GGTAAACAAG AAGTAAGCAG AACCAAGCCT TCACTGCTTA TACCCTCCCA CGCTCAAGGA GAGCTGACCG GACGGGACA TAAATGGTTG
 ACCTGCCACA CCAATTGTTT TCCCTTCTGTC TTGGTCTGCG AGTCACGAAT ATGGGAGGCT GCGAGTTCTCT CTCGACTGGC CTGCCCTCTGT ATTTACCAAC
 prM Hypr

601 E G D S L R T H L T R V E G W V W K N R L L A L A M V T V V W L T L
 GAGGCGACT CACTCGAAC ACATTTGACC CGCTGCGAGG GCTGGGTCTG GAAATACGG CTGTTGGCCC TCGCTATGGT GACAGTCGTT TGGCTCAGCG
 CTCCCGCTGA GTGAGGCTTG TGTAACTGG GCGCAGCTCC CGACCCAGAC CTTTTTAGCC GACAACCGG AGCGATACCA CTGTACGACAA ACCGAGTGGC
 E Hypr

prM Hypr

701 E S V V T R V A V L V V L L C L A P V Y A S R C T H L E N R D F V
 TGGAGTCGT GGTACTCGC GTGGCAGTC TGGTGGTGT CCTCTGCTT GCGCTGTCT AGCGTCCAG GTGTACTCAT TTGGAACAAC GAGATTTGT
 ACCTCAGACA CCAATGAGCG CACGTCAGC ACCACCAGA GAGGACAGAA CCGGACAGA CACGAGTGC CACATGAGTA AACCTTTGT CTCTAAACA
 E Hypr

801 T G T Q G T T R V T L V L E L G G C V T I T A E G K P S M D V W L
 CACCGCACC CAGGGACGA CTCGGGTAAC CTTGGTGTCT GAATGGGTG GTTGGCTAC TATTACCGCT GAGGGCAAC CCTCTATGGA TGTGTGGCTG
 GTGGCCGTGG GTCCCTCTGT GAGCCCATG GGACCACGAA CTGACCCAC CAAAGCAATG ATAAATGGGA CTCGCGTTTG GGAGATACCT ACACACCGAC
 E Hypr

901 D A I Y Q E N P A Q T R E Y C L H A K L S D T K V A A R C P T M G P
 GATGCAATCT ATCAGAGAA TCCCGACAA ACCAGGGAAT ATGCTCTCA CGCAAGCTG TCCGATACAA AGGTGCGCGC TAGGTGCCCA ACAATGGGAC
 CTACGTTAGA TAGTCTCTT AGGGGTGTT TGTCCCTTA TACGGAGT GCGTTTCGAC AGGTATGTT TCCAGCGCGG ATCCACGGGT TGTACCCCTG
 E Hypr

1001 A T L A E E H Q G G T V C K R D Q S D R G W G N H C G L F G K G S
 CGGCCACCT GCGGAGGAA CATCAGGAG GTACAGTGTG CAAACGGAC CAGAGTGATA GAGGTGGG TAATCACTGC GGCTGTTCG GCAAGGAAG
 GCCGGTGGGA CCGCTCTT GTAGTCCCTC CATGTACAC GTTGGCCTG GTCTCACTAT CTCGACCC ATTAGTGAGC CCGGACAAAG CGTTTCCTTC
 E Hypr

1101 I V A C V K A A C E A K K A T G H V Y D A N K I V Y T V K V E P
 TATTGTGCT TCGTCAAG CAGCTGTGA GGCACAAAG AAGCTACTG GGCACCTTA TGACCCCAAC AGATCGTTT ATACAGTGA AGTGGACCA
 ATACAGCGA AGCAGTTCC GTCCGACACT CCGTTTTC TTCCGATGAC CCGTGAGAT ACTCGGTTG TTCTAGCAAA TATGTCACTT TCACCTTGT
 E Hypr

1201 H T G D Y V A A N E T H S G R K T A S F T V S S E K T I L T M G E Y
 CACACGGG ATTACGTGC GGCACACGAG ACTCATTCG GTCCGAAAC GGCACGCTTC ACCGTGTCT CCGAAGAC CATCTCACT ATGGGGAGT
 GTGTGTCCC TAATGCACCG CCGTTTGTCT TGAATAAGC CAGCGTTTG CCGGTGGAAG TGGCACAGTA GGCCTTTCTG GTAGGAGTGA TACCCCTCA
 E Hypr

1301 G D V S L L C R V A S G V D L A Q T V I L E L D K T V E H L P T A
 ATGGCGAGT TTCTGTCTC TCGGGGTGG CTACGGAGT CAGCTGGCC CAGACGTCA TCCGTGAAT GGTAAACA GTTGAATC TGCCTACCG
 TACCGCTGA AAGACAGAG ACAGCCACC GATCGCTCA CACTGTCTG GTCTGTCTG AGGACCTTGA CCTATTTGT CCACTCTAG ACGGATGGC
 E Hypr

1401 . W Q V H R D W F N D L A L P W K H E G A R N W N N A E R L V E F G
TTGCAGGTG CACAGGGATT GGTTTAAGCA CCTTGCCTG CCAATGAAC ATGAAGGAGC GAGAACTGG AATAATGCAG AGCACTCGT AGAATTCGGT
AACCGTCCAC GTGTCCCTAA CCAAATTGCT GGAACGGAC GGTACTTTG TACTTCTCG CTCTTGACC TTATTAGTC TCGTGTAGCA TCTTAAGCCA
E Hypr

1501 A P H A V K M D V Y N L G D Q T G V L L K A L A G V P V A H I E G T
GCCCTCATG CCGTGAAGAT GGACGTCTAC AATCTGGTG ATCAGACCG CGTCTCTCTT AAAGCTCTCG CTGGCTTACC AGTTGCCAC ATCGAAGGAA
CGGGAGTAC GGCACCTTCTA CCGTGCAGAT TTAGACCCAC TAGTCTGGC GCAAGAGGAA TTTCGAGAGC GACCGCATGG TCAACGGTG TAGCTTCTT
E Hypr

1601 . K Y H L K S G H V T C E V G L E K L K M K G L T Y T M C D K T K F
CGAAGTACCA CCTGAAGTCA GGCCATGTAA CTTCGGAGGT GGGCTGGAG AAGTTGAAA TGAAGGTCT TACGTACACA ATGTGTGACA AGACCAAGTT
GCTTCATGTT GGACTTCAGT CCGGTACATT GAACGTCCA CCGGACCTC TTCAACTTTT ACTTTCAGA ATGCATGTGT TACACACTGT TCTGGTTCAA
E Hypr

1701 . T W K R A P T D S G H D T V V M E V T F S G T K P C R I P V R A V
CACATGGAG AGGGCCCCA CAGATAGCGG CCACATACT GTGTGTAGT AGGTGACCTT TTCTGGAACA AAACCTGCA GAATACCCGT GCGGCTGTA
GTATACCTTC TCCCGGGGT GTCTATGCC GGTCTATGA CACCACTACC TCCACTGGA AAGACCTTGT TTGGGACGT CTTATGGGCA CGCCGACAT
E Hypr

1801 A H G S P D V N V A M L I T P N P T I E N N G G F I E M Q L P P G
GCTCAGGAT CTCCCGATGT CAATGTGCT ATGTGATTA CACTTAACC TACCATGAG AATAAGGTG GTGGTTTAT TGAGATGAG CTTCCGCCAG
CGAGTGCCTA GAGGGTACA GTTACAACGA TACGACTAAT GTGATTTGG ATGTAGCTC TTATTGCCAC CACCAAAATA ACTCTAGTC GAAGGCGTTC
E Hypr

1901 . D N I I Y V G E L S Y Q W F Q K G S I G R V F Q K T K K G I E R
GCATAACAT CATCTAGTG GCGAACTCT CTACACAGTG GTTTCAGAAA GGGATTCAA TTGGGCGGT CTTCCAAA ACGAAGAAG GAATCGAAG
CGCTATTGTA GTAGATGCAC CCGTTGAGA GAATGGTTC CAAAGTCTT CCGTCAAGT AACCCGCCA GAAGTTTTT TGCTTCTTCC CTTAGCTTGC
E Hypr

2001 . L T V I G E H A W D F G S A G G F L S S I G K A L H T V L G G A F
ATGACGTT ATCGGCGAGC ACGATGGGA TTTGGTTCC GCAGGGGAT TCGTGTCTC TATTGTAAG GCATGATA CCGTGTGGG GGGCGCATTC
TAACTGCCAA TAGCGCTCG TCGGTACCT AAACCAAGG CGTCCCTCA AGACAGAAG ATACCATTC CGTGACGTAT GGCACGACCC CCGCGTAAAG
E Hypr

2101 N S I F G V G F L P K L L L G V A L A W L G L N M R N P T M S M S
AATCTATT TCGGGGCGT GGGTTCCTG CTTAACTCC TGCTGGAGT AGCCCTGGC TGGTTGGAC TGAATATGCG GAATCCGACG ATGTCCATGT
TTAAGATAAA AGCCCCGCA CCGCAAGGAC GGATTGAGG ACGACCTCA TCGGGACCG ACCAACCTG ACTTATACG CTTAGGCTGC TACAGGTACA
E Hypr

2201 . F L L A G V L V L A M T L G V G A D T G C A I D I S R Q
CATTCCTCTT GCGCGCGTG CTTGTACTGG CCATGACAT GGGCGTGGC CCGGACATG GGTGTGCCAT AGACATCAGC CGGCAA
GTAAGGAGAA CCGGCGGCAC GAACATGACC GGTACTGTGA CCGCAACCG GGGCTGTGAC CCACAGGTA TCTGTAGTGC GCGGTT

WNV NS1 protein

PIV-WN/TBEV Hypr with WNV signal (p40)

	deleted C	5' UTR		

				M S
1	AGTAGTTGCG CTGTGTGAGC TGACAAACTT AGTAGTGTTT GTGAGGATTA ACAACAATTA ACACAGTGG AGCTGTTTCT TAGCACGAAG ATCTCGATGT			
	TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCTTAAT TGTTGTTAAT TGTCACCG TCGACAAAGA ATCGTGTCTC TAGAGTACA			

101	K K P G G P G K S R A V Y L L K R G M P R V L S L I G L K R S S K			
	CTAAGAAC AGAGGGCC GGCAGAGCC GGCCTGCTA TTGTCTAATA CGCGAATGC CCGCGTGT GTCTTGATT GACTTAAAGC GGAGCTCCAA			
	GATTCCTTGG TCCTCCCGG CCGTCTCGG CCGACAGAT AAACGATTTT GCGCTTACG GGGCGCACAA CAGGAACATA CCTGAATTCG CCTCGAGGTT			

		WNV signal		

	WNV deleted C			prM Hypr

201	Q K K R G G K T G I A V M I G M L A C V G A A T V R K E R D G S T			
	GCAAGAAA CGCGGGGAA AGACAGGCAT AGCTGTGATG ATAGCATGC TGGCTGTGT CGGAGCAGCT ACGTGGCA AAGAAGCGA CGGAAGCACC			
	CGTTTCTTT GCGCCCCCTT TCTGTCCGTA TCGACACTAC TATCCGTACG ACCGAACACA GCTCTGCGA TGACACGCTT TCTTGGCT GCCTTCGTGG			

		prM Hypr		

301	V I R A E G K D A A T Q V R V E N G T C V I L A T D M G S W C D D S			
	GTGATAAGG CTGAGGTAA GGTGCGCT AGCAGGTGA GAGTAGAGAA TGGCACTTGC GTAATCTCG CGACTGATAT GGGATCCTGG TGTGACGATA			
	CACTATTCCC GACTCCCAT TCTACGCCA TCGTCCACT CTCACTCTT ACCGTGAAG CATTATGAGC GCTGACTATA CCCTAGGACC ACCTGCTAT			

		prM Hypr		

401	L S Y E C V T I D Q G E E P V D V D C F C R N V D G V Y L E Y G R			
	GCCTCAGTTA TGAATGCGTA ACAATAGACC AGGGCGAAGA ACCTGTGGAC GTTCACTGTT TCTGTAGAA TGTGGATGGC GTTATCTGG AGTAGGGCCG			
	CGGAGTCAAT ACTTAGCAT TGTATCTGG TCCCGCTTCT TGGACACCTG CAACTGACAA AGACATCTTT ACACCTACCG CAAATAGACC TCATGCCGCG			

		prM Hypr		

501	C G K Q E G S R T R R S V L I P S H A Q G E L T G R G H K W L E G			
	CTGTGGAAA CAGAGGGCT CACGAATCG AAGATCTGTG CTGATTCCAA GTACGCGCA AGGAGAGTGG ACCGGTAGAG GCCACAAGTG GCTTGAAGGG			
	GACACCTTTT GTCTCTCCGA GTGCTTGAGC TTCTAGACAC GACTAAGGTT CAGTGGCGT TCCTCTCAAC TGGCCATCTC CGGTGTTTAC CGAATTTCCC			

		prM Hypr		

601	D S L R T H L T R V E G W V W K N R L L A L A M V T V V W L T L E S			
	GACTCATTGA GGRCCCACT GACTAGGTG GAGGTTGGG TTGTGAAGAA TCGGTGCTC GCGTCGCTA TGGTCACCGT CGTGTGGCTG ACCTGGAGA			
	CTGAGTAACT CCGGGTGA CTGATCCAC TCCCAACCC AAACCTTCTT AGCCAACGAG CCGGAGGAT ACCAGTGGCA GCACACCGAC TGTGACCTCT			

		E Hypr		

		prM Hypr		

701	V V T R V A V L V V L L C L A P V Y A S R C T H L E N R D F V T G			
	GTGTGCTGAC TCGGGTTGCT GTGTTGGTTG TCCTCTCTG TTTGGCCCCA GTGTACGGT CCAGGTGTAC TCATTGGAA AACAGAGATT TTGTACCCGG			
	CACAGCACTG AGCCCCACGA CACAACCAAC AGGAGGAGAC AAACCGGGT CACATGGCA GGTCCCATG AGTAAACCTT TTGTCTCTAA AACAGTGGCC			

		E Hypr		

801 . T Q G T T R V T L V L E L G G C V T I T A E G K P S M D V W L D A
CACCGGGG ACAGCTGGG TAACCTGGT GGTGTTGCTG TTACTATTAC CGCTGAGGC AAACCTCTTA TGGATGTG GCTGGATGCA
GTGGGTCCC TGCTAGCCC ATTGGGACCA CGAATTGAC CCACCAAGC AATGATAATG GCGACTCCG TTGGGAGAT ACCTACACAC CGACTACGCT
E Hypr

901 I Y Q E N P A Q T R E Y C L H A K L S D T K V A A R C P T M G P A T
ATCTATCAGG AGAATCCCG ACAACACAGG GAATATTGCC TTACGAAA GCTGTCCGAT ACAAGGTG CGCTAGTG CCCAACATG GGACCGGCCA
TAGATAGTCC TCTTAGGGG TGTGTGGTCC CTATTAAGG AAGTGGTCTT GCACAGGCTA TGTTCCAGC GCGATCCAC GGGTGTGTAC CCTGGCCGGT
E Hypr

1001 . L A E E H Q G G T V C K R D Q S D R G W G N H C G L F G K G S I V
CCCTGCGGA GGAACATCAG GGAGGTACAG TGTGCAACG GGACAGAT GATAGAGCT GGGTAATCA CTGCGGCTG TTGGGCAAG GAAGTATTGT
GGGACCGCT CCTGTAGTC CCTCCATGTC ACAGTTTGC CCGTGTCTCA CTATCTCGA CCCATTAAT GAGCCCGAC AAGCCGTTT CTTCATAACA
E Hypr

1101 . A C V K A A C E A K K K A T G H V Y D A N K I V Y T V K V E P H T
CGTTGCGTC AAGCAGCT GTGAGGCCAA AAGAAGCT ACTGGCAGC TCTATGAGC CAACAAGATC GTTATATACG TGAAGTGA ACCACACACA
GGAACGCAG TTCGTGCGA CACTCCGGT TTCTTCGGA TGACCGTGC AGATACTGC GTTGTCTTAG CAATATATGCT ACTTTCACCT TGGTGTGTGT
E Hypr

1201 G D Y V A A N E T H S G R K T A S F T V S S E K T I L T M G E Y G D
GGGATTACG TGGCGGCCA CGAGACTCAT TCCGGTCCGA AAAGCCAG CTTCACCGTG TCATCCGAAA AGACCACTCT CACTATGGG GAGTATGGC
CCCCTAATGC ACCGCCGTT GCTCTGACTA AGGCCAGCT TTGCGGTC GAATGGCAC AGTAGGCTTT TCTGGTAGGA GTGATACCC CTCATACCGC
E Hypr

1301 . V S L L C R V A S G V D L A Q T V I L E L D K T V E H L P T A W Q
ACGTTTCTCT GCTCTGCGG GTGGTAGG GAGTCAGCT GGCACAGCA GTCATCTGG AACTGGATAA AACAGTTGAG CATCTGCCTA CCGTTGGCA
TGCAAGAGA CGACAGGCC CACCGATGC CTCAGCTGGA CCGGTCTGT CAGTAGGACC TTGACCTATT TTGTCACTC GTAGACGAT GGCGAACCGT
E Hypr

1401 . V H R D W F N D L A L P W K H E G A R N W N N A E R L V E F G A P
GGTGACAGG GATGGTTTA AGACCTTGC CCGCATGG AAACATAG GAGCGAGAA CTGGAATAAT GCAGAGGCAC TCGTAGAATT CGGTGCCCC
CCAGTGTC CTAACCAAT TGCTGGAACG GGACGTACC TTGTACTTC CTCGCTCTT GACCTTATTA CGTCTGCTG AGCATCTTAA GCCACGGGA
E Hypr

1501 H A V K M D V Y N L G D Q T G V L L K A L A G V P V A H I E G T K Y
CATGCCGTA AGATGGAGCT CTACAATCTG GGTATCAGA CCGCGTTCT CCTTAAAGCT CTCGCTGGC TACCAGTGC CCACATCGAA GGAACGAAGT
GTACGGCACT TCTACCTGCA GATGTTAGAC CCACATAGTCT GGCGCAAGA GGAATTGCA GAGCGACGC ATGTCACAG GGTGTAGCTT CCTTGCTTCA
E Hypr

1601 . H L K S G H V T C E V G L E K L K M K G L T Y T M C D K T K F T W
ACCACCTGAA GTCAGGCCAT GTAATTGCG AGGTGGCCT GGAGAGTTG AAATGAAG GTCTACGTA CACAATGTGT GACAAGACCA AGTTACATG
TGGTGGACTT CAGTCCGTA CATTAACGC TCCACCGGA CCTCTTCAAC TTTTACTTTC CAGAATGCAT GTGTTACACA CTGTTCTGCT TCAAGTGATC
E Hypr

1701 . K R A P T D S G H D T V V M E V T F S G T K P C R I P V R A V A H
GAAGAGGCC CCCACAGATA GCGGCCAGA TACTGTGGT ATGAGGTGA CCTTTCTGG AACAAACCC TGCAGATAC CCGTGGGGC TGAGCTCAC
CTTCTCCCG GGGTGTCTAT CGCCGTGCT ATGACACCAC TACTCCACT TACCTCCACT TGTGTTGGG AGGTCTTATG GGCACGCCG ACATCSAGT
E Hypr

1801 G S P D V N V A M L I T P N P T I E N N G G G F I E M Q L P P G D N
GGATCTCCG ATGTCAATGT TGCTATGCTG ATTACACTA ACCCTACCAT CGAGAATAC GGTGGTGTGTT TTATTGAGT GCAGCTCCG CCAGCGGATA
CCTAGAGGC TACAGTTACA AGCATAGAC TAATGTGGAT TGGATGGTA GCTCTTATG CCACCACCA AATACTCTA CGTCGAAGC GGTCCGCTAT
E Hypr

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1901  . I I Y V G E L S Y Q W F Q K G S S I G R V F Q K T K K G I E R L T
      ACATCATCTA CGTGGGCGAA CTCTCTTACC AGTGGTTTCA GAAGGGAGT TCAATTGGC GGTCTTTCCA AAAACGAAG AAGGAATCG AACGATTGAC
      TGTAGTAGAT GCACCCGCTT GAGAGAAATGG TCACCAAGT CTTTCCCTCA AGTTAACCG CCGAGAAGGT TTTTGCTTC TTCCCTTAGC TTGCTAACTG
      E Hypr

2001  . V I G E H A W D F G S A G G F L S S I G K A L H T V L G G A F N S
      GGTATCGGC GAGCAGCAT GGGATTITGG TTCCGACGG GGAITTCCTGT CTTCATTGG TAAGGCACTG CATACCGTGC TGGGGGCGC ATTCATTTCT
      CCAATAGCCG CTCGTGCGTA CCTTAAACC AAGGCTCCC CCTAAGGACA GAAGATAACC ATTCCGTGAC GTATGGCAG ACCCCCCCG TAAGTTAAGA
      E Hypr

2101  . I F G G V G F L P K L L L G V A L A W L G L N M R N P T M S M S F L
      ATTTTCGGG GCGTGGGTTT CCGCCCTAAA CTCCTGCTGG GAGTAGCCCT GGCTGTGTTG GGAAGTGAATA TCGGAATCC GACGATGTC ATGTCATTCC
      TAAAGCCCC CGCACCCCAA GGACGGATTI GAGGACGACC CTCATCGGGA CCGGACCAAC CCTGACTTAT AGCCCTTAGG CTGCTACAGG TACAGTAAGG
      E Hypr

      WNV NSI protein

2201  . L A G V L V L A M T L G V G A D T G C A I D I S R Q
      TCTTGGCCG CGTGCTTGTA CTGGCCATGA CACTGGCGT TGGCCCGAC ACTGGGTGTG CCATAGACAT CAGCCGCAA
      AGAACCGCC GCACGAACAT GACCGGTACT GTGACCCGCA ACCGCGCTG TGACCCACAC GGTATCTGTA GTCGGCGGTT

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Sequence Appendix 4. WN PIV constructs expressing rabies virus G protein.

WN (Δ CprME)-Rabies PIV sequence (partial)

N-terminus of C

5' UTR

M S

1 AGTAGTTCGC CTGTGTGAGC TGACAAACTT AGTAGTGTTC GTGAGGATTA ACAACAATTA ACACAGTGGC AGCTGTTTCT TAGCACGAAG ATCTCGATGT
TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCCTAAT TGTGTGTAAT TGTGTACGCG TCGACAAAGA ATCGTGCTTC TAGAGCTACA
N-terminus of C

K K P G G P G K S R A V Y L L K R G M P R V L S L I G L K Q K K R

101 CTAAGAAACC AGGAGGGCCC GGCAAGAGCC GGGCTGTCTA TTTGCTAAAA CGCGGAATGC CCCGCGTGTG GTCCTTGATT GGACTTAAGC AAAAGAAGCG
GATTCTTTGG TCCTCCCGGG CCGTTCTCGG CCCGACAGAT AAACGATTTT GCGCCTTACG GGGCGCACAA CAGGAACATA CCTGAATTCG TTTTCTTCGC
N-terminus of C Rabies-G signal

partial C signal Rabies-G protein

G G K T G I A V I V P Q A L L F V P L L V F P L C F G K F P I Y T

201 AGGGGGCAAG ACTGGTATAG CTGTGATCGT TCCTCAGGCT CTTTGTGTTG TACCTTGCT GGTATTTCCC CTTGTCTTTG GTAAATTTCC TATCTATACC
TCCCGGCTTC TGACCATATC GACACTAGCA AGGAGTCCGA GAAACAAAC ATGGGAACGA CCATAAAGGG GAAACGAAAC CATTAAAGG ATAGATATGG
Rabies-G protein

I P D K L G P W S P I D I H H L S C P N N L V V E D E G C T N L S G

301 ATCCCTGATA AGCTCGGGCC TTGGAGTCCC ATTGATATTC ACCATTGAG CTGCCCAAAC AACCTCGTCG TTGAGGATGA AGGGTGCAT AATCTTTCTG
TAGGGACTAT TCGAGCCCGG AACCTCAGGG TAACTATAAG TGGTAACTC GACGGGTTTG TTGGAGCAGC AACTCTACT TCCACGTGA TTGAAAGAC
Rabies-G protein

F S Y M E L K V G Y I S A I K M N G F T C T G V V T E A E T Y T N

401 GATTTTCTTA CATGGAGTTG AAAGTGGGCT ATATTTCAGC CATTAAAGATG AACGGCTTTA CTTGTACAGG AGTCGTGACC GAAGCCGAGA CATATACAAA
CTAAAGGAT GTACCTCAAC TTTACCCCGA TATAAAGTCG GTAATCTAC TTGCCGAATG GAACATGTCC TCAGCACTGG CTTGCGCTCT GTATATGTTT
Rabies-G protein

F V G Y V T T T F K R K H F R P T P D A C R A A Y N W K M A G D P

501 TTTCGTGGGA TACGTACCA CCACCTTCAA GAGAAAACAC TTCCGCCCAA CGCCTGACGC TTGTGCGGCC GCTTACAAC TGAAGATGGC AGGAGATCCT
AAAGCACCT ATGCAGTGGT GGTGAAGTT CTCTTTTGTG AAGCGGGTT GCGGACTGCG AACAGCCCGG CGAATGTTGA CTTCTACCG TCCTCTAGGA
Rabies-G protein

R Y E E S L H N P Y P D Y H W L R T V K T T K E S L V I I S P S V A

601 CGATATGAAG AATCTCTGCA CAACCGTAT CCTGATTACC ATTGGCTGCG GACAGTCAAG ACTACCAAGG AGAGTCTGGT CATTATATCA CCAAGCGTGG
GCTATACTTC TTAGAGACGT GTTGGGCATA GGACTAATGG TAAACGAGCG CTGTGAGTTC TGATGGTTCC TCTCAGACCA GTAATATAGT GGTTCGACCC
Rabies-G protein

D L D P Y D R S L H S R V F P G G N C S G V A V S S T Y C S T N H

701 CCGATCTGA TCCTTATGAT AGATCCCTGC ACAGTAGGGT TTTCTCTGGC GGGAAATGTA GCGGTGTTGC AGTATCAAGT ACCTACTGCT CCACTAACCA
GGCTAGAAT AGGAATACTA TCTAGGGACG TGTATCCCA AAAAGGACCG CCCTTAACAT CGCCACAACG TCATAGTTCA TGGATGACGA GGTGATTGGT
Rabies-G protein

D Y T I W M P E N P R L G M S C D I F T N S R G K R A S K G S E T

801 CGACTACACT ATATGGATGC CTGAGAACCC TCGACTCGGT ATGAGTTGCG ACATTTTAC GAACTCACGG GGCAAGCGGG CATCTAAGGG GTCTGAAACA
GCTGATGTA TATACCTACG GACTCTTGGG AGCTGAGCA TACTCAACGC TGTAAATAATG CTTGAGTGCC CCGTTCGCC GTAGATTCCC CAGACTTTGT
Rabies-G protein

C G F V D E R G L Y K S L K G A C K L K L C G V L G L R L M D G T W

901 TGCGGGTTTG TTGATGAGCG GGGGTTGTAT AAATCTCTTA AAGGCGCTG TAAGCTGAAA CTCTGTGGCG TACTGGGGCT GCGCTGATG GACGGCACAT
ACGCCAAAC AACTACTCGC CCCCAACATA TTTAGAGAAT TTCCGCGGAT TTGCACTTT GAGACACCG ATGACCCCGA CGCGGACTAC CTGCGGTGA
Rabies-G protein

V A M Q T S N E T K W C P P G Q L V N L H D F R S D E I E H L V V

1001 GGGTGGCTAT GCAGACAAGC AATGAAACAA AGTGGTGTCC CCCTGGTCAG CTGGTTAATC TGCACGACTT TAGGTCTGAC GAAATCGAGC ACCTTGTGGT
CCCACGATA CGTCTGTTCC TTACTTTGTT TCACCAACAG GGGACAGTC GACCAATTAG ACGTGCTGAA ATCCAGACTG CTTTAGCTCG TGGAACACCA
Rabies-G protein

	· E E L V K K R E E C L D A L E S I M T T K S V S P R R L S H L R K ·
1101	GGAGGAAC TG GTGAAGAAAC GCGAAGAGTG CCTGGACGCA CTTGAGAGTA TTATGACCAC CAAATCCGTT TCCTTCAGAA GACTGAGCCA CCTGCGAAAG CCTCCTTGAC CACTTCTTTG CGTTCTTCAC GGACCTGCGT GAACTCTCAT AATACTGGTG GTTTAGGCAA AGGAAGTCTT CTGACTCGGT GGACGCTTTC Rabies-G protein
	· L V P G F G K A Y T I F N K T L M E A D A H Y K S V R T W N E I I P ·
1201	CTGGTGCCAG GGTTCGGGAA GGCTTATACT ATTTTCAACA AGACTCTTAT GGAGGCGGAT GCCCATTATA AGTCAGTTAG GACTTGGAAT GAGATAATTC GACCACGGTC CCAAGCCCTT CCGAATATGA TAAAAGTTGT TCTGAGAATA CCTCGCCTA CGGGTAATAT TCAGTCAATC CTGAACCTTA CTCTATTAAG Rabies-G protein
	· S K G C L R V G G R C H P H V N G V F F N G I I L G P D G N V L I ·
1301	CCTCCAAAGG ATGTCTGAGA GTCGGTGGGA GATGCCACCC CCATGTCAAT GGGGTGTTCT TTAACGGAAT CATCCTGGGA CCTGACGGGA ACGTGTCTGAT GGAGGTTTCC TACAGACTCT CAGCCACCCT CTACGGTGGG GGTACAGTTA CCCACAAGA AATTGCCTTA GTAGGACCCT GGACTGCCCT TGCACGACTA Rabies-G protein
	· P E M Q S S L L Q Q H M E L L V S S V I P L M H P L A D P S T V F ·
1401	TCCGAGATG CAATCTTCCC TTCTGCAGCA ACACATGGAA CTCCTGGTGT CTTCACTGAT ACCCTGATG CACCCACTGG CCGACCCAG CACTGTGTTC AGGCTCTAC GTTAGAAGG AAGACGTCGT TGTGTACCTT GAGGACCACA GAAGTCACTA TGGGGACTAC GTGGGTGACC GGCTGGGGTC GTGACACAAG Rabies-G protein
	· K N G D E A E D F V E V H L P D V H E R I S G V D L G L P N W G K Y ·
1501	AAAAATGGCG ATGAGGCCGA AGACTTTGTG GAAGTTCACC TGCCGATGT ACACGAAAGG ATATCTGGAG TAGACCTGGG CCTTCCTAAT TGGGGTAAAGT TTTTTACCGC TACTCCGGCT TCTGAAACAC CTTCAAGTGG ACGGGCTACA TGTGCTTTCC TATAGACCTC ATCTGGACCC GGAAGGATTA ACCCCATTCA Rabies-G protein
	· V L L S A G A L T A L M L I I F L M T C W R R V N R S E P T Q H N ·
1601	ACGTGCTCCT GAGTGGGGT GCCTTGACCG CTTTGATGCT GATCATTTT CTTAGTACCT GCTGGCGGAG GGTGAATCGC TCCGAGCCGA CACAGCACAA TGACGAGGA CTCACGCCCA CGAACTGGC GAAACTACGA CTAGTAAAAA GACTACTGGA CGACCGCTC CCACTTAGCG AGGCTCGGCT GTGTCTGTGT Rabies-G protein
	----- FMDV 2A -----
	· L R G T G R E V S V T P Q S G K I I S S W E S Y K S G G E T G L N ·
1701	TCTCAGAGGG ACAGGCCGGG AAGTAAGTGT GACTCCGCAA TCTGGCAAGA TTATTAGTAG TTGGGAGAGT TACAAGTCTG GAGGAGAGAC TGGGTGAAT AGAGTCTCCC TGTCCGGCCC TTCATTACA CTGAGGCGTT AGACCGTTCT AATAATCATC AACCTCTCA ATGTTAGAGC CTCCTCTCTG ACCCAACTTA preNS1 signal
	----- FMDV 2A ----- NS1 signal -----
	· F D L L K L A G D V E S N P G P A R D R S I A L T F L A V G G V L L ·
1801	TTTGATCTGC TCAAACTTGC AGGCGATGTA GAATCAAATC CTGGACCGCG CCGGACAGG TCCATAGCTC TCACGTTTCT CGCAGTTGGA GGAGTTCTGC AAACTAGAGC AGTTTGAACG TCCGCTACAT CTTAGTTTAG GACTGGGCGG GGCCTGTCC AGGTATCGAG AGTGCAAAGA GCGTCAACCT CCTCAAGACG NS1 signal
	----- NS1 -----
	· F L S V N V H A D T G C A I D I S R Q E L R C G S G V F I H N D V ·
1901	TCTTCCTCTC CGTGAACGTG CACGCTGACA CTGGGTGTGC CATAGACATC AGCCGGCAAG AGCTGAGATG TGGAAGTGA GTGTTTCATC ACAATGATGT AGAAGGAGAG GCACCTGCAC GTGCGACTGT GACCCACAG GTATCTGTAG TCGGCCGTTT TCGACTCTAC ACCTTCACCT CACAAGTATG TGTTACTACA

WN (Δ C)-Rabies G PIV sequence (partial).

5'UTR

terminus of C

N-

M S

1 AGTAGTTGCG CTGTGTGAGC TGACAACTT AGTAGTGTTC GTGAGGATTA ACAACAATTA ACACAGTGCG AGCTGTTTCT TAGCAGGAAG ATCTCGATGT
TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCCTAAT TGTTGTTAAT TGTGTCACGC TCGACAAAGA ATCGTGCTTC TAGAGCTACA
N-terminus of C

K K P G G P G K S R A V N M L K R G M P R V L S L I G L K Q K K R

101 CTAAGAAACC AGGAGGGCCC GGCAAGAGCC GGGCTGTCAA TATGCTAAAA CGCGGAATGC CCGCGTGTT GTCTTGATT GGACTTAAGC AAAAGAAGCG
GATTCTTTGG TCCTCCCGGG CCGTTCTCGG CCGACAGTT ATACGATTTC GCGCCTTACG GGGCGCACAA CAGGAACATA CCTGAATTGG TTTTCTTCGC
N-terminus of C

Rabies-G protein

partial C signal Rabies-G signal

G G K T G I A V I V P Q A L L F V P L L V F P L C P G K F P I Y T

201 AGGGGGCAAG ACTGGTATAG CTGTGATCGT TCCTCAGGCT CTTTGTGTTG TACCTTGCT GGTATTTCCT CTTGCTTTG GTAAATTTCC TATCTATACC
TCCCGCGTTC TGACCATATC GACACTAGCA AGGAGTCCGA GAAACAAAC ATGGGAACGA CCATAAAGG GAAACGAAAC CATTAAAGG ATAGATATGG
Rabies-G protein

I P D K L G P W S P I D I H H L S C P N N L V V E D E G C T N L S G

301 ATCCCTGATA AGCTCGGGCC TTGGAGTCCC ATTGATATTC ACCATTTGAG CTGCCCAAAC AACCTCGTCG TTGAGGATGA AGGGTGCACT AATCTTTCTG
TAGGGACTAT TCGAGCCCGG AACCTCAGGG TAACTATAAG TGAATTTCTAC TTGCCGAAAT TTGGAGCAGC AACTCCTACT TCCCACGTGA TTAGAAGAC
Rabies-G protein

F S Y M E L K V G Y I S A I K M N G P T C T G V V T E A E T Y T N

401 GATTTTCTTA CATGGAGTTG AAAGTGGGCT ATATTTCAGC CATTAAGATG AACGGCTTTA CTGTACAGG AGTCGTGACC GAAGCCGAGA CATATACAAA
CTAAAAGGAT GTACCTCAAC TTCACCCGA TATAAAGTCG GTAATTTCTAC TTGCCGAAAT GAACATGTCC TCAGCACTGG CTTCGGCTCT GTATATGTTT
Rabies-G protein

F V G Y V T T T P K R K H F R P T P D A C R A A Y N W K M A G D P

501 TTTCGTGGGA TACGTCACCA CCACCTTCAA GAGAAAACAC TTCGCCCCAA CGCCTGACGC TTGTCGGGCC GCTTACAACCT GGAAGATGGC AGGAGATCCT
AAGCACCTC ATGCAGTGGT GGTGGAAGTT CTCTTTTGTG AAGGCGGGTT GCGGACTGCG AACAGCCCGG CGAATGTGA CCTTCTACCG TCCTCTAGGA
Rabies-G protein

R Y E E S L H N P Y P D Y H W L R T V K T T K E S L V I I S P S V A

601 CGATATGAAG AATCTCTGCA CAACCCGTAT CCTGATTACC ATTGGCTGCG GACAGTCAAG ACTACCAAGG AGAGTCTGGT CATTATATCA CCAAGCGTGG
GCTATACCTC TTAGAGACGT GTTGGGCATA GGACTAATGG TAAACGACGC CTGTCACTTC TGATGGTTCC TCTCAGACCA GTAATATAGT GGTTCGCACC
Rabies-G protein

D L D P Y D R S L H S R V F P G G N C S G V A V S S T Y C S T N H

701 CCGATCTGA TCCTTATGAT AGATCCCTGC ACAGTAGGGT TTTTCTGGC GGGAATTGTA GCGGTGTTGC AGTATCAAGT ACCTACTGCT CCACTAACCA
GGCTAGAACT AGGAATACTA TCTAGGGACG TGTCATCCCA AAAAGGACCG CCCTTAACAT CGCCACAACG TCATAGTTCA TGGATGACGA GGTGATTGGT
Rabies-G protein

D Y T I W M P E N P R L G M S C D I F T N S R G K R A S K G S E T

801 CGACTACACT ATATGGATGC CTGAGAACCC TCGACTCGGT ATGAGTTGCG ACATTTTAC GAACTCACGG GGCAAGCGGG CATCTAAGGG GTCTGAAACA
GCTGATGTGA TATACCTACG GACTCTTGGG AGCTGAGCCA TACTCAACGC TGTAAAAATG CTTGAGTGCC CCGTTCCGCC GTAGATTCCC CAGACTTTGT
Rabies-G protein

C G F V D E R G L Y K S L K G A C K L K L C G V L G L R L M D G T W

901 TGCGGGTTTG TTGATGAGCG GGGGTTGTAT AAATCTCTTA AAGGCGCCTG TAAGCTGAAA CTCTGTGGCG TACTGGGGCT GCGCCTGATG GACGGCACAT
ACGCCCAAAC AACTACTCGC CCCCACATA TTAGAGAAT TTCCGCGGAC ATTCGACTTT GAGACACCGC ATGACCCCGA CGCGGACTAC CTGCCGTGTA
Rabies-G protein

V A M Q T S N E T K W C P P G Q L V N L H D F R S D E I E H L V V

1001 GGGTGGCTAT GCAGACAAGC AATGAAACAA AGTGGTGTCC CCTGGTCTAG CTGGTTAATC TGACAGACTT TAGGTCTGAC GAAATCGAGC ACCTTGTGGT
CCCACCGATA CGTCTGTTCG TTACTTTGTT TCACCACAGG GGGACCACTG GACCAATTAG ACGTGTCTGAA ATCCAGACTG CTTTAGCTCG TGGAACACCA
Rabies-G protein

E E L V K K R E E C L D A L E S I M T T K S V S F R R L S H L R K

1101 GGAGGAACTG GTGAAGAAAC GCGAAGAGTG CCTGGACGCA CTTGAGAGTA TTATGACCAC CAAATCCGTT TCCTTCAGAA GACTGAGCCA CCTGCGAAAG
CCTCCTTGAC CACTTCTTTG CGTTCTCAC GGACCTGCGT GAACTCTCAT AATACTGGTG GTTTAGGCAA AGGAAGTCTT CTGACTCGGT GGACGCTTTC
Rabies-G protein

L V P G F G K A Y T I F N K T L M E A D A H Y K S V R T W N E I I P .

1201 CTGGTGCCAG GGTTCGGGAA GGCTTATACT ATTTTCAACA AGACTCTTAT GGAGGCGGAT GCCCATTATA AGTCAGTTAG GACTTGGAAT GAGATAATTC
GACCACGGTC CCAAGCCCTT CCGAATATGA TAAAGTTGT TCTGAGAATA CCTCCGCCTA CGGTAATAT TCAGTCAATC CTGAACCTTA CTCTATTAAG
Rabies-G protein

· S K G C L R V G G R C H P H V N G V F F N G I I L G P D G N V L I .

1301 CCTCCAAAGG ATGTCTGAGA GTCGGTGGGA GATGCCACCC CCATGTCAAT GGGGTGTTCT TTAACGGAAT CATCCTGGGA CCTGACGGGA ACGTGCTGAT
GGAGGTTTCC TACAGACTCT CAGCCACCCT CTACGGTGGG GGTACAGTTA CCCACAAGA AATTGCCTTA GTAGGACCCT GGACTGCCCT TGCACGACTA
Rabies-G protein

· P E M Q S S L L Q Q H M E L L V S S V I P L M H P L A D P S T V F .

1401 TCCCGAGATG CAATCTTCCC TTCTGCAGCA ACACATGGAA CTCCTGGTGT CTTCACTGAT ACCCCTGATG CACCCACTGG CCGACCCAG CACTGTGTTT
AGGGCTCTAC GTTAGAAGG AAGACGTCGT TGTGTACCTT GAGGACCACA GAAGTCACCTA TGGGGACTAC GTGGGTGACC GGCTGGGGTC GTGACACAAG
Rabies-G protein

K N G D E A E D F V E V H L P D V H E R I S G V D L G L P N W G K Y .

1501 AAAAATGGCG ATGAGGCCGA AGACTTTGTG GAAGTTCACC TGCCCGATGT ACACGAAAGG ATATCTGGAG TAGACCTGGG CCTTCTTAAT TGGGGTAAAGT
TTTTTACCG TACTCCGGCT TCTGAAACAC CTTCAAGTGG ACGGGCTACA TGTGCTTTCC TATAGACCTC ATCTGGACCC GGAAGGATTA ACCCAATTCA
Rabies-G protein

· V L L S A G A L T A L M L I I F L M T C W R R V N R S E P T Q H N .

1601 ACGTGCTCCT GAGTGCGGGT GCCTTGACCG CTTTGATGCT GATCATTITT CTGATGACCT GCTGGCGGAG GGTGAATCGC TCCGAGCCGA CACAGCACAA
TGACAGAGGA CTCACGCCCA CGGAACCTGGC GAAACTACGA CTAGTAAAAA GACTACTGGA CGACCGCCTC CCACCTAGCG AGGCTCGGCT GTGTCTGTGT
FMDV 2A

Rabies-G protein

· L R G T G R E V S V T P Q S G K I I S S W E S Y K S G G E T G L N .

1701 TTCTCAGAGG ACAGGCCGGG AAGTAAGTGT GACTCCGCAA TCTGGCAGA TTATTAGTAG TTGGGAGAGT TACAAGTCTG GAGGAGAGAC TGGGTGAAT
AGAGTCTCCC TGTCCGGCCC TTCATTACA CTGAGGCGTT AGACCGTTCT AATAATCATC AACCTCTCA ATGTTTCAGAC CTCCTCTCTG ACCCAACTTA
C/prM signal

FMDV 2A

F D L L K L A G D V E S N P G P G G K T G I A V M I G L I A C V G A .

1801 TTTGATCTGC TCAAACTTGC AGGCGATGTA GAATCAAATC CTGGACCCGG AGGAAAGACC GGTATTGCAG TCATGATTGG CCTGATCGCC TGCCTAGGAG
AAACTAGACG AGTTTGAACG TCCGCTACAT CTTAGTTTAG GACTTGGGCC TCCTTTCTGG CCATAACGTC AGTACTAACC GGACTAGCGG ACGCATCTCT
C/prM signal

prM

· V T L S N F Q G K V M M T V N A T D V T D V I T I P T A A G K N L .

1901 CAGTTACCCT CTCTAACTTC CAAGGGAAGG TGATGATGAC GGTAAATGCT ACTGACGTCA CAGATGTCAT CACGATTCCA ACAGTGTCTG GAAAGAACCT
GTCAATGGGA GAGATTGAAG GTTCCCTTCC ACTACTACTG CCATTACGA TGACTGCAGT GTCTACAGTA GTGCTAAGGT TGTGACGAC CTTTCTTGGG
prM

· C I V R A M D V G Y M C D D T I T Y E C P V L S A G N D P E D I D .

2001 ATGCATTGTC AGAGCAATGG ATGTGGGATA CATGTGCGAT GATACTATCA CTTATGAATG CCCAGTGTCT TCGGCTGGTA ATGATCCAGA AGACATCGAC
TACGTAACAG TCTCGTTACC TACACCTAT GTACACGCTA CTATGATAGT GAATACTTAC GGGTCACGAC AGCCGACCAT TACTAGGTCT TCTGTAGCTG
prM

C W C T K S A V Y V R Y G R C T K T R H S R R S R R S L T V Q T H G .

2101 TGTGTGTGCA CAAAGTCAGC AGTCTACGTC AGGTATGGAA GATGCACCAA GACACGCCAC TCAAGACGCA GTCGGAGGTC ACTGACAGTG CAGACACAG
ACAACCAAGT GTTTCAGTCG TCAGATGCAG TCCATACCTT CTACGTGGTT CTGTGCGGTG AGTTCTGCGT CAGCCTCCAG TGACTGTAC GTCTGTGTGC
prM

· E S T L A N K K G A W M D S T K A T R Y L V K T E S W I L R N P G .

2201 GAGAAAGCAC TCTAGCGAAC AAGAAGGGGG CTTGGATGGA CAGCACCAAG GCCACAAGGT ATTTGGTAAA AACAGAATCA TGGATCTTGA GGAACCTTGG
CTCTTTCGTG AGATCGCTTG TTCTTCCCCC GAACCTACCT GTCGTGGTTC CGGTGTTCCTA TAAACCATTT TTGTCTTAGT ACCTAGAAGT CCTTGGGACC
prM

· Y A L V A A V I G W M L G S N T M Q R V V F V V L L L L V A P A Y .

2301 ATATGCCCTG GTGGGACCGC TCATTGGTTG GATGCTTGGG AGCAACACCA TGCAGAGAGT TGTGTTTGTG GTGCTATTGC TTTTGGTGGC CCCAGCTTAC
TATACGGGAC CACCGTCGGC AGTAACCAAC CTACGAACCC TCGTTGTGGT AGCTCTCTCA ACACAACAG CAGGATAACG AAAACCAACG GGGTCGAATG
E

prM

S F N C L G M S N R D F L E G V S G A T W V D L V L E G D S C V T I .

2401 AGCTTTAACT GCCTTGAAT GAGCAACAGA GACTTCTTGG AAGGAGTGTG TGGAGCAACA TGGGTGGATT TGGTTCTCGA AGGCGACAGC TGCCTGACTA
TCGAAATTGA CGGAACCTTA CTCGTTGTCT CTGAAGAACC TTCTCAGAC ACCTCGTTGT ACCCACTAA ACCAAGAGCT TCCGCTGTG ACGCACTGAT
E

· M S K D K P T I D V K M M N M E A A N L A E V R S Y C Y L A T V S ·
2501 TCATGTCTAA GGACAAGCCT ACCATCGATG TGAAGATGAT GAATATGGAG GCGGCCAACC TGGCAGAGGT CCGCAGTTAT TGCTATTGGG CTACCGTCAG
AGTACAGATT CCTGTTCCGA TGGTAGCTAC ACTTCTACTA CTTATACCTC CGCCGGTTGG ACCGTCTCCA GGCGTCAATA ACGATAAACC GATGGCAGTC
E
· D L S T K A A C P A M G E A H N D K R A D P A F V C R Q G V V D R ·
2601 CGATCTCTCC ACCAAAGCTG CGTGCCCGGC CATGGGAGAA GCTCACAATG ACAAACGTGC TGACCCAGCT TTTGTGTGCA GACAAGGAGT GGTGGACAGG
GCTAGAGAGG TGGTTTCGAC GCACGGGCGG GTACCCTCTT CGAGTGTGTAC TGTTTGACAG ACTGGGTGCA AAACACACGT CTGTCTCTCA CCACCTGTCC
E
· G W G N G C G L F G K G S I D T C A K F A C S T K A I G R T I L K E ·
2701 GGCTGGGGCA ACGGCTGCGG ACTATTGGC AAAGGAAGCA TTGACACATG CGCCAAATTT GCCTGCTCTA CCAAGGCAAT AGGAAGAACC ATTTTGAAAG
CGACCCCGT TGCCGACGCC TGATAAACCG TTTCTTCTGT AACTGTGTAC GCGGTTTAAA CGGACGAGAT GGTTCCTGTA TCCTTCTTGG TAAAACTTTC
E
· N I K Y E V A I F V H G P T T V E S H G N Y S T Q V G A T Q A G R ·
2801 AGAATATCAA GTACGAAGTG GCCATTTTGG TCCATGGACC AACTACTGTG GAGTCGCACG GAAACTACTC CACACAGGTT GGAGCCACTC AGGCAGGGAG
TCTTATAGTT CATGCTTCAC CGGTAAAAAC AGGTACCTGG TTGATGACAC CTCAGCGTGC CTTTGATGAG GTGTGTCCAA CCTCGGTGAG TCCGTCCTTC
E
· F S I T P A A P S Y T L K L G E Y G E V T V D C E P R S G I D T N ·
2901 ATTCAGCATC ACTCTGCGG CGCCTTCATA CACACTAAAG CTGGGAGAAT ATGGAGAGGT GACAGTGGAC TGTGAACCAC GGTGAGGATG TGACACCAAT
TAAGTCGTAG TGAGGACGCC GCGGAAGTAT GTGTGATTTC GAACCTCTTA TACCTCTCCA CTGTACCTG ACACCTGGTG CCAGTCCCTA ACTGTGGTTA
E
· A Y Y V M T V G T K T F L V H R E W F M D L N L P W S S A G S T V W ·
3001 GCATACTACG TGATGACTGT TGGAAACAAAG ACGTTCTTGG TCCATCGTGA GTGGTTCATG GACCTCAACC TCCCTTGGAG CAGTGCTGGA AGTACTGTGT
CGTATGATGC ACTACTGACA ACCTTGTTTC TGCAAGAACC AGGTAGCACT CACCAAGTAC CTGGAGTTGG AGGGAACCTC GTCACGACCT TCATGACACA
E
· R N R E T L M E P E E P H A T K Q S V I A L G S Q E G A L H Q A L ·
3101 GGAGGAACAG AGAGACGTTA ATGGAGTTTG AGGAACCACA CGCCACGAAG CAGTCTGTGA TAGCATTGGG CTCACAAGAG GGAGCTCTGC ATCAAGCTTT
CCTCTTGTG TCTCTGAAT TACCTCAAAC TCCTTGGTGT GCGGTGCTTC GTCAGACACT ATCGTAACCC GAGTGTCTCT CCTCGAGACG TAGTTCGAAA
E
· A G A I P V E F S S N T V K L T S G H L K C R V K M E K L Q L K G ·
3201 GGCTGGAGCC ATTCTGTGG AATTTTCAAG CAACACTGTC AAGTTGACGT CGGGTCATTT GAAGTGTAGA GTGAAGATGG AAAAATTGCA GTTGAAGGGA
CCGACCTCGG TAAGGACACC TTAAGGTTTC GTGTGACAG TTCAACTGCA GCCCAGTAAA CTTACATCT CACTTCTACC TTTTAAAGT CAACCTCCCT
E
· T T Y G V C S K A F K F L G T P A D T G H G T V V L E L Q Y T G T D ·
3301 ACAACCTATG GCGTCTGTTT AAAGGCTTTC AAGTTTCTTG GGAAGTCCGC AGACACAGGT CACGGCACTG TGGTGTGGGA ATTGCAGTAC ACTGGCACGG
TGTTGGATAC GCGAGACAAG TTTCCGAAAG TTCAAAGAAC CCTGAGGGCG TCTGTGTCCA GTGCCGTGAC ACCACAACCT TAAGTCATG TGACCGTGCC
E
· G P C K V P I S S V A S L N D L T P V G R L V T V N P F V S V A T ·
3401 ATGGACCTTG CAAAGTTCTT ATCTCGTCAG TGGCTTCATT GAACGACCTA ACGCCAGTGG GCAGATTGGT CACTGTCAAC CCTTTTGTG CAGTGGCCAC
TACCTGGAAC GTTCAAGGA TAGAGCAGTC ACCGAAGTAA CTGTCTGGAT TCGGTTCACC CGTCTAACCA GTGACAGTTG GGAACACAA GTACCGGTG
E
· A N A K V L I E L E P P F G D S Y I V V G R G E Q Q I N H H W H K ·
3501 GGCCAACGCT AAGTCTCTGA TTGAATTGGA ACCACCTTTT GGAGACTCAT ACATAGTGGT GGGCAGAGGA GAACAACAGA TCAATCACCA CTGGCACAAG
CCGTTGCGA TTCCAGGACT AACTTAACCT TGGTGGGAAA CCTCTGAGTA TGTATCACCA CCGTCTCCT CTGTGTGTCT AGTTAGTGGT GACCGTGTTC
E
· S G S S I G K A F T T T L K G A Q R L A A L G D T A W D F G S V G G ·
3601 TCTGGAAGCA GCATTGGCAA AGCCTTTTACA ACCACCCTCA AAGGAGCGCA GAGACTAGCC GCTCTAGGAG ACACAGCTTG GACTTTTGGG TCAGTTGGAG
AGACCTTCGT CGTAACCGTT TCGGAAATGT TGGTGGGAGT TTCTCTGCGT CTCTGATCGG CGAGATCCTC TGTGTGCAAC CCTGAAACCT AGTCAACCTC
E
· V F T S V G K A V H Q V F G G A F R S L F G G M S W I T Q G L L G ·
3701 GGGTGTTCAC CTCAGTTGGG AAGGCTGTCC ATCAAGTGTT CGGAGGAGCA TTCCGCTCAC TGTTCGGAGG CATGTCTGGG ATAACGCAAG GATTGCTGGG
CCCACAAGTG GAGTCAACCC TTCCGACAGG TAGTTCACAA GCCTCCTCGT AAGGCGAGTG ACAAGCCTCC GTACAGGACC TATTGCGTTC CTAACGACCC
E
· A L L L W M G I N A R D R S I A L T F L A V G G V L L F L S V N V ·
3801 GGCTCTCCTG TGTGGATGG GCATCAATGC TCGTGACAGG TCCATAGCTC TCACGTTTCT CGCAGTTGGA GGAGTTCTGC TCTTCTCTC CGTGAACGTG
CCGAGAGGAC AACACCTACC CGTAGTTACG AGCACTGTCC AGGTATCGAG AGTGCAAGA GCGTCAACCT CCTCAAGACG AGAAGGAGAG GCACTTGCAC
E

NS1

H A D T G C A I D I S R Q E L R C G S G V F I H N D V E A W M D R Y .

3901 CACGCTGACA CTGGGTGTGC CATAGACATC AGCCGGCAAG AGCTGAGATG TGGAAAGTGA GTGTTTCATAC ACAATGATGT GGAGGCTTGG ATGGACCGGT
GTGCGACTGT GACCCACACG GTATCTGTAG TCGGCCGCTT TCGACTCTAC ACCTTCACCT CACAAGTATG TGTTACTACA CCTCCGAACC TACCTGGCCA

WN (Δ prME)-Rabies G PIV sequence (partial)

C protein

5' UTR

M S .

1 AGTAGTTCGC CTGTGTGAGC TGACAACTT AGTAGTGTTC GTGAGGATTA ACAACAATTA ACACAGTGC AGCTGTTTCT TAGCACGAAG ATCTCGATGT
TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCCTAAT TGTTGTTAAT TGTGTCACGC TCGACAAAGA ATCGTGCTTC TAGAGCTACA
C protein

· K K P G G P G K S R A V Y L L K R G M P R V L S L I G L K R A M L ·

101 CTAAGAAACC AGGAGGGCCC GGCAAGAGCC GGGCTGTCTA TTTGCTAAAA CGCGGAATGC CCCGCGTGTG GTCCTTGATT GGACTTAAGA GGGCTATGTT
GATTCTTTGG TCCTCCCGGG CCGTTCTCGG CCGACAGAT AAACGATTTT GCGCCTTACG GGGCGCACAA CAGGAAGTAA CCTGAATTCT CCGGATACAA
C protein

· S L I D G K G P I R F V L A L L A F F R F T A I A P T R A V L D R ·

201 GAGCTGATC GACGGCAAGG GGCAATACG ATTTGTGTTG GCTCTCTTGG CGTTCTTCAG GTTCACAGCA ATTGCTCCGA CCGAGCAGT GCTGGATCGA
CTCGGACTAG CTGCCGTTCC CCGTTATGTC TAAACACAAC CGAGAGAACC GCAAGAAGTC CAAGTGTGCT TAACGAGGCT GGGCTCGTCA CGACCTAGCT
C protein

W R G V N K Q T A M K H L L S F K K E L G T L T S A I N R R S S K Q ·

301 TGGAGAGGTG TGAACAAACA AACAGCGATG AAACACCTTC TGAGTTTCAA GAAGGAACTA GGGACCTTGA CCAGTGTCTAT CAATCGGCGG AGCTCAAAGC
ACCTCTCCAC ACTGTGTTGT TTGTCGCTAC TTGTGGAAG ACTCAAAGTT CTTCTTGAT CCCTGGAAGT GGTACAGATA GTTAGCGGCC TCGAGTTTCG
Rabies-G signal

C protein partial C signal Rabies-G
protein

· K K R G G K T G I A V I V P Q A L L F V P L L V F P L C F G K F P ·

401 AAAAGAAGCG AGGGGGCAAG ACTGGTATAG CTGTGATCGT TCCTCAGGCT CTTTGTGTTG TACCTTGCT GGTATTTCCC CTTTGCTTTG GTAAATTTCC
TTTCTTCGC TCCCCGTTT TGACCATATC GACACTAGCA AGGAGTCCGA GAAACAAAC ATGGGAACGA CCATAAAGG GAAACGAAAC CATTTAAAGG
Rabies-G protein

· I Y T I P D K L G P W S P I D I H H L S C P N N L V V E D E G C T ·

501 TATCTATACC ATCCCTGATA AGCTCGGGCC TTGGAGTCCC ATTGATATTC ACCATTGAG CTGCCCAAAC AACCTCGTCG TTGAGGATGA AGGGTGCACT
ATAGATATGG TAGGGACTAT TCGAGCCCGG AACCTCAGG TAACATAAG TGGTAAACTC GACGGGTTG TTGAGCAGC AACTCCTACT TCCCAGTGTA
Rabies-G protein

N L S G F S Y M E L K V G Y I S A I K M N G F T C T G V V T E A E T ·

601 AATCTTTCTG GATTTTCTTA CATGGAGTTG AAAGTGGGCT ATATTTTCAGC CATTAAGATG AACGGCTTTA CTGTACAGG AGTCGTGACC GAAGCCGAGA
TTAGAAAGAC CTAAGAGGAT GTACCTCAAC TTTCACCGA TATAAAGTCG GTAATTCTAC TTGCCGAAAT GAACATGTCC TCAGCACTGG CTTCGGCTCT
Rabies-G protein

· Y T N F V G Y V T T T F K R K H F R P T P D A C R A A Y N W K M A ·

701 CATATACAAA TTTCGTGGGA TACGTCACCA CCACCTTCAA GAGAAAACAC TTCGCCCCAA CGCTGACGC TTGTCGGGCC GCTTACAAC TGAAGATGGC
GTATATGTTT AAAGCACCTT ATGCACTGGT GGTGGAAGTT CTCTTTGTG AAGGCGGGTT GCGGACTGCG AACAGCCCGG CGAATGTTGA CCTTCTACCG
Rabies-G protein

· G D P R Y E E S L H N P Y P D Y H W L R T V K T T K E S L V I I S ·

801 AGGAGATCCT CGATATGAAG AATCTCTGCA CAACCCGTAT CCTGATTACC ATTGGCTGCG GACAGTCAAG ACTACCAAGG AGAGTCTGGT CATTATATCA
TCCTCTAGGA GCTATACTTC TTAGAGACGT GTTGGGCATA GGACTAATGG TAACCGACGC CTGTCAGTTC TGATGGTTCC TCTCAGACCA GTAATATAGT
Rabies-G protein

P S V A D L D P Y D R S L H S R V F P G G N C S G V A V S S T Y C S ·

901 CCAAGCGTGG CCGATCTTGA TCCTTATGAT AGATCCCTGC ACAGTAGGGT TTTTCTGGC GGAATTTGTA GCGGTGTTGC AGTATCAAGT ACCTACTGCT
GGTTCGCACC GGCTAGAACT AGGAATACTA TCTAGGGACG TGTCATCCCA AAAAGGACCG CCCTTAACAT CGCCACAACG TCATAGTTCA TGGATGACGA
Rabies-G protein

· T N H D Y T I W M P E N P R L G M S C D I F T N S R G K R A S K G ·

1001	CCACTAACCA CGACTACACT ATATGGATGC CTGAGAACCC TCGACTCGGT ATGAGTTGCG ACATTTTAC GAACTCACGG GGCAAGCGGG CATCTAAGGG GGTGATTGGT GCTGATGTGA TATACCTACG GACTCTTGGG AGCTGAGCCA TACTCAACGC TGTAATAATG CTTGAGTGCC CGTTGCGCC GTAGATTCCTC Rabies-G protein
	· S E T C G F V D E R G L Y K S L K G A C K L K L C G V L G L R L M ·
1101	GTCGTAAACA TGCGGGTTTG TTGATGAGCG GGGGTTGTAT AAATCTCTTA AAGGCGCCTG TAAGCTGAAA CTCTGTGGCG TACTGGGGCT GCGCTGATG CAGACTTTGT ACGCCCAAAC AACTACTCGC CCCCACATA TTTAGAGAAT TTCCGCGGAC ATTGACTTTT GAGACACCGC ATGACCCCGA CGCGGACTAC Rabies-G protein
	· D G T W V A M Q T S N E T K W C P P G Q L V N L H D F R S D E I E H ·
1201	GACGGCACAT GGTGGCTAT GCAGACAAGC AATGAAACAA AGTGGTGTC CCCTGGTCAG CTGGTTAATC TGCACGACTT TAGGTCTGAC GAAATCGAGC CTGCCGTGTA CCCACCGATA CGTCTGTTTG TACTTTTGT TTACACACAGG GGGACCACTG GACCAATTAG ACGTGCTGAA ATCCAGACTG CTTAGCTCG Rabies-G protein
	· L V V E E L V K K R E E C L D A L E S I M T T K S V S F R R L S H ·
1301	ACCTTGTTGGT GGAGGAAGT GTGAAGAAAC GCGAAGAGTG CCTGGACGCA CTTGAGAGTA TTATGACCAC CAAATCCGTT TCCTTCAGAA GACTGAGCA TGGAACACCA CTCCTTGAC CACTTCTTGT CGCTTCTCAC GGACCTGCGT GAATCTCAT AATACTGGTG GTTAGGCAA AGGAAGTCTT CTGACTCGGT Rabies-G protein
	· L R K L V P G F G K A Y T I F N K T L M E A D A H Y K S V R T W N ·
1401	CCTGCGAAAG CTGGTGCCAG GGTTCGGGAA GGCTTATACT ATTTTCAACA AGACTCTTAT GGAGGCGGAT GCCATTATA AGTCAGTTAG GACTTGGAAT GGACGCTTTC GACCACGGTC CCAAGCCCTT CCGAATATGA TAAAGTTGT TCTGAGAATA CCTCCGCCTA CGGTAATAT TCAGTCAATC CTGAACCTTA Rabies-G protein
	· E I I P S K G C L R V G G R C H P H V N G V F F N G I I L G P D G N ·
1501	GAGATAATTC CCTCCAAAGG ATGTCTGAGA GTCGGTGGGA GATGCCACCC CCATGTCAAT GGGGTGTTCT TTAACGGAAT CATCTGGGA CCTGACGGGA CTCTATTAAG GGAGGTTTCC TACAGACTCT CAGCCACCCT CTACGGTGGG GGTACAGTTA CCCCACAAGA AATTGCTTCA GTAGGACCCT GGACTGCCCT Rabies-G protein
	· V L I P E M Q S S L L Q Q H M E L L V S S V I P L M H P L A D P S ·
1601	ACGTGCTGAT TCCCGAGATG CAATCTTCCC TTCTGCAGCA ACACATGGAA CTCTGGTGT CTTAGTGAT ACCCTGATG CACCCACTGG CCGACCCAG TGACGACTA AGGGCTCTAC GTTAGAAGGG AAGACGTCGT TGTGTACCTT GAGGACCACA GAAGTCACTA TGGGGACTAC GTGGGTGACC GGCTGGGTC Rabies-G protein
	· T V F K N G D E A E D F V E V H L P D V H E R I S G V D L G L P N ·
1701	CACTGTGTTT AAAATGGCG ATGAGGCCGA AGACTTTGTG GAAGTTCACC TGCCCGATGT ACACGAAAGG ATATCTGGAG TAGACCTGGG CCTTCCTAAT GTGACACAAG TTTTACCOC TACTCCGCT TCTGAAACAC CTTCAAGTGG ACGGCTACA TGTGCTTTC TATAGACCTC ATCTGGACCC GGAAGGATTA Rabies-G protein
	· W G K Y V L L S A G A L T A L M L I I F L M T C W R R V N R S E P T ·
1801	TGGGTAAGT ACGTGCTCTT GAGTGCGGT GCCTTGACCG CTTTGATGCT GATCATTTT CTGATGACCT GCTGGCGGAG GGTGAATCGC TCCGAGCGA ACCCATTCA TGCACGAGGA CTCAGCCCCA CGGAAGTGGC GAAACTACGA CTAGTAAAAA GACTACTGGA CGACCGCCTC CCACTTAGCG AGGCTCGGCT Rabies-G protein
	· Q H N L R G T G R E V S V T P Q S G K I I S S W E S Y K S G G E T ·
1901	CACAGACAA TCTCAGAGG ACAGGCCGGG AAGTAAGTGT GACTCCGCAA TCTGGCAAGA TTATTAGTAG TTGGGAGAGT TACAAGTCTG GAGGAGAGAC GTGTCGTGTT AGAGTCTCCC TGTCGGGCC TCCATTGACA CTGAGGCGTT AGACGTTCT AATAATCATC AACCTCTCA ATGTTTCAGC CTCCTCTCTG FMDV 2A NS1 signal
	· G L N F D L L K L A G D V E S N P G P A R D R S I A L T F L A V G ·
2001	TGGGTTGAAT TTTGATCTGC TCAAACTTGC AGGCGATGTA GAATCAAATC CTGGACCCGC CCGGGACAGG TCCATAGCTC TCACGTTTCT CGCAGTTGGA ACCCAACTTA AAACAGAGC AGTTTGAACG TCCGCTACAT CTTAGTTTAG GACCTGGGCG GGCCCTGTCC AGGTATCGAG AGTGCAAAGA GCGTCAACCT NS1
	· G V L L F L S V N V H A D T G C A I D I S R Q E L R C G S G V F I H ·
2101	GGAGTTCTGC TCTTCTCTC CGTGAACGTG CACGCTGACA CTGGGTGTGC CATAGACATC AGCCGGCAAG AGCTGAGATG TGAAGTGA GTGTTTCATC CCTCAAGACG AGAAGGAGAG GCACTTGACG GTGCGACTGT GACCCACACG GTATCTGTAG TCGGCCGTTT TCGACTCTAC ACCTTCACCT CACAAGTATG

Sequence Appendix 4 (continued)

PIV-WNV helper ΔNS1

5' UTR

C

1 AGTAGTTCCG CTGTGTGAGC TGACAAACTT AGTAGTGTTC GTGAGGATTA ACAACAATTA ACACAGTGG AGCTGTTTCT TAGCAGGAAG ATCTCGATGT
TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCTAAT TGTGTTAAT TGTGTACGC TCGACAAGA ATCGTGCTTC TAGAGCTACA
C

101 K K P G G P G K S R A V N M L K R G M P R V L S L I G L K R A M L
CTAAGAAACC AGGAGGGCCC GGCAAGAGCC GGGCTGTCAA TATGTAATA CGCGAATGC CCGCGTGTTC GTCTTGATT GGACTTAAGA GGGCTATGTT
GATTTCTTTC TCCTCCCGGG CCGTTCTCGG CCGACAGTT ATACGATTTT GCGCTTACG GGGCGCACAA CAGGAATAA CCTGAATICT CCGGATACAA
C

201 S L I D G K G P I R F V L A L L A F F R F T A I A P T R A V L D R
GAGCTGATC GAGGCAAGG GGCCAATACG ATTGTGTG GCTCTCTGG CGTCTCTCAG GTTCACAGCA ATTGCTCCGA CCCGAGCAGT GCTGGATCGA
CTCGGACTAG CTGCCGTTCC CCGGTTATGC TAAACACAAC CGAGAGAACC GCAAGAAGTC CAAGTGTCTG TAACGAGGCT GGGCTCGTCA CGACCTAGCT
C

301 W R G V N K Q T A M K H L L S F K K E L G T L T S A I N R R S S K Q
TGGAGAGGTG TGAACAACA AACAGCGATG AAACACCTTC TGAGTTTCAA GAAGNACTA GGGACCTTGA CCAGTCTAT CAATCGCGCG AGCTCAAAAC
ACCTCTCCAC ACTTGTTTGT TTGTGCTAC TTGTGGAAG ACTCAAAGTT CTTCCTTGAT CCTGGAAT GGTACAGATA GTTAGCGCC TCGAGTTTTC
Signal peptide

401 K K R G G K T G I A V M I G L I A S V G A V T L S N F Q G K V M M
AAAGAAAG AGGAGGAAG ACCGGAATG CAGTCATGAT TGGCTGATC GGCAGGTAG GGCAGTTAC CCTCTAAC TTCCAAGGA AGGTGATGAT
TTTCTCTTTC TCCTCCTTTC TGGCCTTAC GTCAGTACTA ACCGACTAG CCGTGGCATC CTGTCATG GAGAGATTG AAGGTTCCCT TCCACTACTA
prM

501 T V N A T D V T D V I T I P T A A G K N L C I V R A M D V G Y M C
GACGTAAAT GCTACTGAGC TCACAGATGT CATCAGATT CCAACAGTGT CTGGAAGAA CCTATGCATT GTCAGAGCAA TGGATGTGG ATACATGTC
CTGCCAATTA CGATGACTGC AGTGTCTACA GTAGTGCTAA GGTGTGCTAC GACCTTCTT GGTATAGTAA CAGTCTCGTT ACCTACACCC TATGTACACG
prM

601 D D T I T Y E C P V L S A G N D P E D I D C W C T K S A V Y V R Y G
GATGATACTA TCACCTATGA ATGCCAGTG CTGTGGCTG GTATGATCC AGAAGACATC GACTGTGGT GCACAAGTC AGCAGTCTAC GTACAGGTATG
CTACTATGAT AGTGAATACT TACGGGTAC GACAGCCGAC CATTACTAGG TCTTCTGTAG CTGACAACCA CGTGTTCAG TCGTCAGATG CAGTCCATAC
prM

701 R C T K T R H S R R S R S L T V Q T H G E S T L A N K K G A W M
GAAGATGCAC CAAGACAGC CACTCAAGAC GCAGTCGGAG GTCACTGACA GTGAGACAC ACAGGAAG CACTCTAGCG AACAGAAG GGGCTTGGAT
CTTCTACGTC GTTCTGTGCG GTGAGTTCTG CGTCAGCCTC CAGTGACTGT CAGTCTCTTC GTGAGATCGC TTGTTCTTCC CCGGAACCTA
prM

prM

801 . D S T K A T R Y L V K T E S W I L R N P G Y A L V A A V I G W M L
GGACAGCACC AAGGCCACAA GGTATTGGT AAAACAGAA TCATGGATCT TGAGGAACCC TGGATATGCC CTGGTGGCAG CCGTCAATGG TTGGATGCTT
CCTGTGCTGG TTCCGGTGT CCATAAACCA TTTTGTCTT AGTACTAGA ACTCCTTGGG ACCTATACGG GACCACCGTC GGCAGTAACC AACCTACGAA
E

prM

901 G S N T M Q R V V F V V L L L L V A P A Y S F N C L G M S N R D F L
GGGAGCAACA CCATGCAGAG AGTTGTGTTT GTCTGTCTAT TGCTTTGGT GGGCCACGCT TACAGCTTTA ACTGCTTGG AATGAGCAAC AGAGACTTCT
CCCTCGTTGT GGTACGTCTC TCAACACAAA CAGCAGGATA ACGAAAACCA CCGGGGTGGA ATGTCGAAAT TGACGGAACC TTACTCGTTG TCTCTGAAGA
E

1001 . E G V S G A T W V D L V L E G D S C V T I M S K D K P T I D V K M
TGAAGGAGT GTCTGGAGCA ACATGGGTGG ATTTGTTCT CGAAGGCGAC AGTGCCTGA CTATCATGTC TAAGACAAG CCTACCATCG ATGTGAAGAT
ACCTTCCTCA CAGACCTCGT TGTACCACC TAAACCAAGA GCTCCGCTG TCGACGCACT GATGTACAG ATTCTGTTC GGATGGTAGC TACACTTCTA
E

1101 . M N M E A A N L A E V R S Y C Y L A T V S D L S T K A A C P A M G
GATGAATAG GAGCGGCCA ACCTGGCAGA GGTCCGCGT TATGTCTATT TGGCTACGCT CAGCGATCTC TCCACCAAAG CTGCGTGCCC GGCCATGGGA
CTACTTATAC CTCGCGCGGT TGGACCGTCT CCAGCGTCA ATACGATAA ACCGATGGCA GTCGCTAGAG AGTGGTTTC GACGACGGG CCGGTACCGT
E

1201 E A H N D K R A D P A F V C R Q G V V D R G W G N G C G L F G K G S
GAGCTCACA ATGACAAACG TGCTGACCA GCTTTGTGT GCAGACAGG AGTGGTGGAC AGGGGTGGG GCAACGGCTG CGACTATTT GGCAGGAA
CTTCGAGTGT TACTGTTTGC ACGACTGGGT CGAAAACACA CGTCTGTTC TCACCACCTG TCCCCGACC CGTTCCGAC GCCTGATATA CCGTTTCCTT
E

1301 . I D T C A K F A C S T K A I G R T I L K E N I K Y E V A I F V H G
GCATTGACAC ATGCGCCAAA TTTGCTGCT CTACAAGC AATAGGAAGA ACCATTTTGA AAGAGATAT CAAGTACGAA GTGGCCATTT TTGTCCATGG
CGTAACCTGT TAGCGGTTT AAACGGACGA GATGGTCCG TTATCTCTTCT TGTATAAACT TTCTCTATA GTTCATGCTT CACCGGTAAC AACAGGTACC
E

1401 . P T T V E S H G N Y S T Q V G A T Q A G R F S I T P A A P S Y T L
ACCAACTACT GTGGAGTCG ACGGAACTA CTCACACAG GTTGGAGCA CTCAGGCGG GAGATTGAGC ATCACTCCTG CGGCGCCTTC ATACACACTA
TGGTTGATGA CACCTCAGG TGCCTTTGAT GAGGTGTGTC CAACCTCGGT GAGTCCGCTC CTCTAAGTCG TAGTGAGGAC GCGCGGGAAG TATGTGTGAT
E

1501 K L G E Y G E V T V D C E P R S G I D T N A Y Y V M T V G T K T F L
AAGCTTGAG AATATGGAGA GGTGACAGT GACTGTGAAC CAGCGTCAAG GATTGACACC AATGATACCT ACGTATGAC TGTGGAACA AAGACGTTCT
TTGGAACCTC TTATACCTCT CCACGTGCAC CTGACACTTG GTGCCAGTCC CTAACCTGG TTACGTATGA TGCATACTG ACAACCTTGT TTCTGCAAGA
E

1601 . V H R E W F M D L N L P W S S A G S T V W R N R E T L M E F E E P
TGGTCCATCG TGAGTGTTC ATGACCTCA ACCTCCCTTG GAGCAGTGT GGAAGTACTG TGTGGAGGAA CAGAGACG TTAATGGAGT TTGAGGAACC
ACCAGGTAGC ACTACCAAG TACCTGGAGT TGGAGGGAAC CTGCTCAGA CTTTCATGAC ACACCTCCTT GTCTCTCTGCA AATTACCTCA AACTCCTTGG
E

1701 . H A T K Q S V I A L G S Q E G A L H Q A L A G A I P V E F S S N T
ACACGCCACG AAGCAGTCTG TGATAGCAAT GGGCTCACAA GAGGGAGCTC TGCATCAGC TTTGGCTGGA GCCATTCTC TGGAAATTTTC AAGCAACACT
TGTGCGGTGC TTCGTCAGAC ACTATCGTAA CCCAGTGT TTCCCTCGAG ACGTAGTTCG AAACCGACCT CGGTAAGGAC ACCTTAAAG TTGCTTGTGA
E

1801 V K L T S G H L K C R V K M E K L Q L K G T T Y G V C S K A F K F L
GTCAAGTTGA CGTCGGGTCA TTGAAGTGT AGAGTGAAGA TGAATAATT GCAGTTGAAG GGAACAACCT ATGGCGTCTG TTCAAAAGCT TTCAAGTTTC
CAGTTCAACT GCAGCCAGT AAACCTTACA TCTCACTTCT ACCTTTTAA CGTCAACTTC CTTGTTGGA TACCGCAGAC AAGTTTCCGA AAGTTCAAAG
E

1901 G T P A D T G H G T V V L E L Q Y T G T D G P C K V P I S S V A S
TTGGGACTCC CGCAGACACA GGTACGGCA CTGTGGTGT GGAATTGCAG TACATGGCA CCGATGGACC TTGCAAGTT CTAATCTCGT CAGTGGCTTC
AAACCTGAGG CGCTGTGTGT CCAGTGCCGT GACACCACAA CCTTAGGTC ATGTGACCGT GCCTACCTGG AACGTTTCAA GGATAGACA GTCACCGAAG
E

2001 L N D L T P V G R L V T V N P F V S V A T A N A K V L I E L E P P
ATTGAACGAC CTAACGCCAG TGGCAGATT GGTCACTGTC AACCTTTTG TTTCAGTGGC CACGGCCAAC GCTAAGGTCC TGATTGAATT GGAACCAACC
TAACTTGCTG GATTGGGTC ACCGCTCTAA CCAGTGACAG TTGGGAAAC AAGTCACCG GTGCGGTTG CGATTCCAGG ACTAACTTAA CCTTGGTGGG
E

2101 F G D S Y I V V G R G E Q Q I N H H W H K S G S S I G K A F T T T L
TTTGAGACT CATACATAGT GGTGGGCGA GGAGAACAAAC AGATCAATCA CCAGTGGCAC AAGTCTGAA GCAGCATGG CAAGCCTTT ACAACCAACC
AAACCTCTGA GTATGTATCA CCACCCGTCT CCTCTTGTG TCTAGTAGT GGTGACCGTG TTCAGACCTT CGTCGTAACC GTTTCGAAA TGTGTTGGG
E

2201 K G A Q R L A A L G D T A W D F G S V G G V F T S V G K A V H Q V
TCAAGGAGC GCAGAGACTA GCCGCTCTAG GAGACACAGC TTGGGACTTT GGATCAGTTG GAGGGTGT CACCTCAGT GGAAGGCTG TCCATCAAGT
AGTTTCTCG CGTCTCTGAT CGCGGAGATC CTCTGTGTG AACCTGAAA CCTAGTCAAC CTCCCCACAA GTGGAGTCAA CCTTCCGAC AGGTAGTTCA
E

2301 F G G A F R S L F G G M S W I T Q G L L G A L L L W M G I N A R D
GTTGCGAGGA GCAITCCGCT CACTGTTGG AGGCAATGCC TGGATAAGC AAGATTGCT GGGGCTCTC CTGTTGGA TGGGATCAA TGCTCGTGAC
CAAGCTCTCT CGTAAGGCGA GTGACAAGCC TCCGTACAGG ACCTATTGG TTCTTAACGA CCCCCGAGAG GACAACACCT ACCCGTAGTT ACGAGCACTG
deleted NS1

E

2401 R S I A L T F L A V G G V L L F L S V N V H A D T G I H R G P A T R
AGGTCCATAG CTCTCACGTT TCTCGCAGTT GGAGGAGTTC TGCTCTTCT CTCCGTGAAC GTGCAAGCTG ACACCTGGAT CCACCGTGA CCTGCCACTC
TCCAGGTATC GAGAGTGCA AGAGCGTCAA CCTCCTCAAG ACAGAAAGGA GAGGCACTTG CAGTGGCAG TGTGACCTA GGTGGCACCT GGACGGTGAG
deleted NS1

2501 T T T E S G K L I T D W C C R S C T L P P L R Y Q T D S G C W Y G
GCACCACCAG AGAGAGCGGA AAGTTGATAA CAGATTGGTG CTGCAAGGAGC TGCACTTAC CACCACTGCG CTACCAAACT GACAGCGCT GTTGGTATGG
CGTGGTGGTG TCTCTCGCT TTCAACTATT GTCTACCAAC GAGTCTCTCG ACGTGAATG GTGGTGAGC GATGGTTGA CTGTGCGCA CAACCATACC
deleted NS1

NS2A

2601 M E I R P Q R H D E K T L V Q S Q V N A Y N A D M I D P F Q L G L
TATGAGATC AGACCACAGA GACATGATGA AAAGCCCTC GTGAGTCAAC AAGTGAATGC TTATAATGCT GATATGATG ACCCTTTCA GTTGGGCTTT
ATACCTTAG TCTGGTGTCT CTGTACTACT TTCTTGGGAG CAGCTCAGT TCTACTTAG AATATTAGA CTATACTAAC TGGGAAAAGT CAACCCGGAA

Sequence Appendix 5

PIV-WNV(Δ prME)/RSV-F

```

      C protein
      ----
      5' UTR
      -----
1   AGTAGTTGCG CTGTGTGAGC TGACAACTT AGTAGTGTTC GTGAGGATTA ACAACAATTA ACACAGTGGC AGCTGTTTCT TAGCAGGAAG ATCTCGATGT
   TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCACAAA CACTCTTAAT TGTTGTTAAT TGTGTACGC TCGACAAAGA ATCGTGCTTC TAGAGCTACA
      C protein
      -----
101  . K K P G G P G K S R A V Y L L K R G M P R V L S L I G L K R A M L .
      CTAGAAAACC AGGAGGGCCC GGCAAGAGCC GGGCTGCTA TTGCTATAA CGCGGAATGC CCGCGGTGTT GTCTTGATT GGACTTAAGA GGGCTATGTT
      GATTCCTTGG TCCTCCCGG CCGTCTCGG CCCGACAGAT AACGATTTT GCGCCTTAGG GGGCGCACAA CAGGAATAA CCTGAATTCT CCGGATACAA
      C protein
      -----
201  . S L I D G K G P I R F V L A L L A F F R F T A I A P T R A V L D R
      GAGCTGATC GACGGCAAGG GGCCAATACG ATTGTGTTG GCTCTCTGG CGTCTTCAG GTTCACAGCA ATTGCTCCGA CCCGAGCAGT GCTGGATCGA
      CTCGGACTAG CTGCCGTTCC CCGGTTATGC TAAACACAAC CGAGAGAACC GCAGAAGTC CAAGTGTGCT TAACGAGGCT GGGCTCGTCA CGACCTAGCT
      NS3 cleavage
      ----
      C protein
      -----
301  W R G V N K Q T A M K H L L S F K K E L G T L T S A I N R R S S K Q .
      TGGAGAGGTG TGAACAACA AACAGCGATG AAACACTTC TGAGTTTCAA GAAGGAATA GGGACCTTGA CCAGTGTAT CAATCGGCGG AGCTCAAAGC
      ACCTCTCCAC ACTTGTTGT TGTCGCTAC TTGTGGAAG ACTCAAAGTT CTTCCTTGAT CCTGGAAT GGTACAGATA GTTAGCGCC TCGAGTTTCG
      F signal
      -----
      NS3 cleavage
      ----
      F1
      -----
401  . K K R G G E L L I L K A N A I T T I L T A V T F C F A S G Q N I T .
      AAAAGAAGCG AGGGGGCGAG TTGCTAATCC TCAAGCAAA TGCAATTACC ACAATCCTCA CTGCAGTCAC ATTTGTTTTT GCTTCTGGT AAAACATCAC
      TTTTCTTCGC TCCCCCGCTC AACGATTAGG AGTTTCGTTT ACGTTAATGG TGTTAGGAGT GACGTCAGTG TAACACAAA CGAAGACCAG TTTTGTAGTG
      F1
      -----
501  . E E F Y Q S T C S A V S K G Y L S A L R T G W Y T S V I T I E L S
      TGAGAATTT TATCAATCAA CATGCAGTGC AGTTAGCAAA GGCTATCTTA GTGCTCTGAG AACTGTTGG TATACAGTG TTATAACTAT AGAATTAAGT
      ACTTCTTAAA ATAGTTAGTT GTACGTCACG TCAATCGTTT CCGATAGAAT CAGGAGACTC TTGACCAACC ATATGTCAC AATATTGATA TCTTAATTCA
      F1
      -----
601  N I K E N K C N G T D A K V K L I K Q E L D K Y K N A V T E L Q L L .
      AATATCAAGG AAAATAGTG TAATGGAACA GATGTAAGG TAAATTTGAT AAAACAAGAA TTAGATAAAT ATAAATATGC TGTAAACAGAA TTGCAGTTGC
      TTATAGTTCC TTTTATTTCAC ATTACCTTGT CTACGATTCC ATTTAACTA TTTTGTCTT AATCTATTTA TATTTTACG ACATGTCTT AACGTCAACG
      F1
      -----
701  . M Q S T P P T N N R A R R E L P R F M N Y T L N N A K K T N V T L .
      TCATGCAAG CACACCACCA ACAACAATC GAGCAGAGG AGAATACCA AGTTTATGA ATTATACACT CAACATGCC AAAAACAACA ATGTAACATT
      AGTACGTTTC GTGTGTGGT TGTTGTAG CTGCTCTTC TCTGTAGTGT TCCAAATACT TAATATGTA GTTGTACGG TTTTTTGTGT TACATTGTAA
      F1
      -----

```

F2

801 . S K K R K R R F L G F L L G V G S A I A S G V A V S K V L H L E G
AAGCAAGAAA AGGAAAGRA GATTTCTGG TTTTGTGTTA GGTGTGGAT CTGCAATCGC CAGTGGCGTT GCTGTATCTA AGGTCTGCA CCTAGAAGG
TTCTGTTCTTT TCCTTTTCTT CTAAGAACC AAAAACAAT CCACACCTA GACGTAGCG GTCCACGCAA CGACATAGAT TCCAGGACGT GGATCTTCCC
F2

901 E V N K I K S A L L S T N K A V V S L S N G V S V L T S K V L D L K
GAAGTGAACA AGATCAAAAG TGCTCTACTA TCCACAACA AGGTGTAGT CAGCTTATCA AATGGAGTTA GTGTCTTAAC CAGCAAGTG TTAGACCTCA
CTTCACTTGT TCTAGTTTTC ACAGATGAT AGGTGTGTGT TCCGACATCA GTCCGAATAGT TTAGCTCAAT CACAGAATG GTCTGTTTTC AATCTGGAGT
F2

1001 . N Y I D K Q L L P I V N K Q S C S I S N I E T V I E F Q Q K N N R
AAAATATAT AGATAAACA TGTGTACTA TTGTACTA GCAAGCTGC AGCATATCAA ATATAGAAC TGTGTATAG TGTGTATAG TGTGTATAG TGTGTATAG
TTTTGATATA TCTATTGT AACAATGAT AACACTTGT CGTTGCGAG TCGTATAGT TATATCTTTC ACATATCTC AAGGTGTGTT TCTGTGTGTC
F2

1101 . L L E I T R E F S V N A G V T T P V S T Y M L T N S E L L S L I N
ACTACTAGAG ATTACCAGG AATTAGTGT TAATGAGGT GTAATGAGT CTGTAAGCAC TTATAGGTTA ACTAATAGT AATTATGTC AATTATCAAT
TGATGATCTC TAATGCTCC TTAATCACA ATTACGTCCA CATTGATG GACATCTG AATGTACAAT TGATTATCAC TTAATAACAG TAATTAGTTA
F2

1201 D M P I T N D Q K K L M S N N V Q I V R Q Q S Y S I M S I I K E E V
GATATGCTTA TAACAAATGA TCAGAAAAG TTAATGTCCA AATAGTTTCA AATAGTTTGA CAGCAAGTT ACTCTATCAT GTCCATAATA AAAGAGGAG
CTATACGGAT ATTGTTTACT AGTCTTTTTC AATTACAGT TGTTACAAGT TTATCAATCT GTCTTTTCAA TGAGATAGTA CAGGTATTAT TTTCTCTTTC
F2

1301 . L A Y V V Q L P L Y G V I D T P C W K L H T S P L C T T N T K E G
TCTTAGCATA TGTAGTACAA TTACCACTAT ATGGTGTAT AGATACACC TGTGTGGAAC TACACATC CCTCTATGT ACAACCAACA CAAAGAAGG
AGAATCGTAT ACATCATGTT AATGGTGATA TACCACAATA TCTATGTGGG ACAACCTTTC AATGTGTAG GGGAGATACA TGTGTGTGT GTTTCTTTC
F2

1401 . S N I C L T R T D R G W Y C D N A G S V S F F P Q A E T C K V Q S
GTCCAACATC TGTTAACAA GNACTGACAG AGGATGGTAC TGTGACAAAG CAGGATCAGT ATCTTTCTTC CCACAGCTG AAACATGTAA AGTTCAATCA
CAGGTGTAG ACAATTTGTT CTGACTGTC TCCTACCATG ACATGTTTAC GTCTTAGTCA TAGAAGAAG GGTGTGCGAC TTTGTACAT TCAAGTTAGT
F2

1501 N R V F C D T M N S L T L P S E I N L C N V D I F N P K Y D C K I M
AATCGAGTAT TTTGTGACAC AATGAACAGT TTAACATTAC CAATGAAT AATCTCTGC AATGTGACA TATTCACCC CAATATGAT TGTAAATTA
TTAGCTCATA AAACACTGTG TTAATGTGTA AATTGTAATG GTTCACTTTA TTTAGAGAGG TTAACAAGT ATAAGTTGG GTTTATATA ACAATTTAAT
F2

1601 . T S K T D V S S S V I T S L G A I V S C Y G K T K C T A S N K N R
TGACTTCRAA AACAGATGTA AGCAGCTCCG TTATCACATC TCTAGGACG ATGTGTGTCAT GCTATGGCAA AACTAATGT ACAGATCCA ATAAATCG
ACTGAAGTTT TTGTCTACAT TCGTCGAGC AATAGTGTAG AGATCTCTCG TTAACACAGTA CGATACCGT TTGAATTACA TGTCTAGGT TATTTTTCAG
F2

1701 . G I I K T F S N G C D Y V S N K G M D T V S V G N T L Y Y V N K Q
TGAATCATA AGACATTTT CTAACGGGTG CGATTATGTA TCAATAAG GATGAGAC TGTGTCTGTA GGTAACAT TATATTATGT AAATAAGCAA
ACCTTAGTAT TTCTGTAAA GATTGCCAC GCTAATACAT AGTTATTTTC CCTACCTGTG ACACAGAT CCATTGTGTA ATATAACA TTTATTCGTT
F2

1801 E G K S L Y V K G E P I I N F Y D P L V F P S D E F D A S I S Q V N
GAAGGTAAA GTCTCTATGT AAAGGTGAA CCAATAATAA ATTTCTATGA CCCATTAGTA TTCCCCTCTG ATGAATTTGA TGCATCAATA TCTCAAGTCA

CTTCCATT T CAGAGATACA TTTTCCACTT GGTATTATT TAAAGATACT GGGTAATCAT AAGGGGAGAC TACTTAACT ACGTAGTTAT AGAGTTTCAGT
F2

TM Domain

1901 . E K I N Q S L A F I R K S D E L L H N V N A G K S T T N I M I T T .
ACGAGAAGAT TAACAGAGC CTAGCAITTA TTCGTAATC CGATGAATTA TTACATAATG TAAATGCTGG TAAATCCACC ACAATATCA TGATAACTAC
TGCTCTTCTA ATTGGTCTCG GATCGTAAT AAGCAITTAG GCTACTTAAT AATGTATTAC ATTACGACC ATTACGACC TGTATTATAGT ACTATTGATG
TM Domain

Cytoplasmic Tail

2001 . I I I V I I V I L L S L I A V G L L L Y C K A R S T P V T L S K D
TATAATTATA GTGATTATAG TAATATTGTT ATCATTAATT GCTGTGGAC TGCTCTTATA CTGTAAGCC AGAAGCACAC CAGTCACACT AAGCAAGAT
ATATTAATAT CACTAATATC ATTATAACAA TAGTAATTAA CGACAACCTG ACGAGAATAT GACATTCCGG TCTTCGTGTG GTCAGTGTGA TTCGTTTCTA
FMDV 2A

Transmembrane

domain of WNV E (split)

Cytoplasmic Tail

2101 Q L S G I N I A F S N N F D L L K L A G D V E S N P G P A R D R S .
CAACTGAGTG GTATAAATAA TATTGCAITT AGTAACAAAT TTGATCTGCT CAACTTGCA GGCATGTAG AATCAAAATCC TGGACCGCC CGGGACAGGT
GTTGACTCAC CATATTATT ATTACGTAAA TCATTGTTAA AACTAGACA GTTTGAACGT CCGCTACATC TTAGTTTAGG ACCTGGGCGG GCCCTGTCCA
NS1

Transmembrane domain of WNV E (split)

2201 . I A L T F L A V G G V L L F L S V N V H A D T G C A I D I S R Q
CCATAGCTCT CACGTTTCTC GCAGTTGGAG GAGTTCTGCT CTTCCTCTCC GTGACGTGC ACGCTGACAC TGGGTGTGCC ATAGACATCA GCCGGCAA
GGTATCGAGA GTGCAAGAG CGTCAACCTC CTCAGACGA GAAGGAGAG CACTTGCACG TGGACTGTG ACCACACAGG TATCTGTAGT CGGCCGTT

PIV-WNV(Δ CprME)/RSV-F

deleted C protein

5' UTR

1 AGTAGTTGCG CTGTGTGAGC TGACAAACTT AGTAGTCTTT GTGAGGATTA ACAACAATTA ACACAGTGG AGTGTTTCT TAGCAGGAG ATCTGATGT
TCATCAAGCG GACACACTCG ACTGTTTGAA TCATCAAAA CACTCTAAT TGTTGTAAT TGTTGTAAT TGTTGTAAT TGTTGTAAT TGTTGTAAT TGTTGTAAT
deleted C protein
M S

NS3 cleavage

. K K P G G P G K S R A V N M L K R G M P R V L S L I G L K Q K K R .

101 CTAAGAAACC AGGAGGCC GGCAGAGCC GGGCTGTCAA TATGCTAAAA CCGCGAATGC CCCGCGTGT GTCTTGATT GGACTTAAGC AAAAGAAGCG
 GATTCCTTGG TCTCCCGG CCGTCTCGG CCCGACAGT ATAGATTIT GCGCCTTAG GCGCGCACAA CAGGAATAA CCTGAATCG TTTTCTTCGC

 F signal

 NS3 cleavage

 F1

 201 AGGGGGCAG TTGCTAATCC TCAAGCAAA TGCAATTACC ACAATCCTCA CTGCAGTCAC ATTTGTTTT GTCTTGTC AAAACATCAC TGAAGAAATT
 TCCCCGCTC AACGATTAGG AGTTTCGTT ACGTAAATGG TGTAGGAGT GAGTCAGT TAAACAAAA CGAAGACCAG TTTTGTAGT ACTTCTTAA

 F1

 301 Y Q S T C S A V S K G Y L S A L R T G W Y T S V I T I E L S N I K E
 TATCAATCAA CATGCAGTC AGTTAGCAA GGCTATCTTA GTGCTCTGAG AACTGGTTGG TATACCAGT TTATAACTAT AGAATTAAGT AATATCAAGG
 ATAGTTAGT GTACGTCAG TCAATCGTT CCGATAGAT CACGAGACTC TTGACCAACC ATATGTCAC AATATTGATA TCTTAATTCA TTATAGTTCC

 F1

 401 N K C N G T D A K V K L I K Q E L D K Y K N A V T E L Q L L M Q S
 AAATAAGTG TAATGGAACA GATGCTAAG TAAATTTGAT AAAACAAGAA TTAGATAAAT ATAAAATGC TGTACAGAA TTGCAGTTGC TCAATCAAG
 TTTTATTCAC ATTACCTGT CTACGATTCC ATTTAACTA TTTTGTCTT AATCTATTA TATTTTACG ACATTTCTT AACGTCACAG AGTACGTTTC

 F1

 501 T P T N N R A R R E L P R F M N Y T L N N A K K T N V T L S K K
 CACCACCA ACAACAATC GAGCCAGAG AGAATACCA AGTTTATGA ATTATACAT CAACATGCC AAAAACAACA ATGTAACAT AAGCAAGAAA
 GTGGTGGT TGTGTGTAG CTCGGTCTTC TCTTGATGT TCCAATACT TAATATGGA GTTGTTACG TTTTGTGGT TACATTGTAA TTCGTTCTTT

 F1

 601 R K R R F L G F L L G V G S A I A S G V A V S K V L H L E G E V N K
 AGGAAAGAA GATTCCTGG TTTTGTGTTA GGTGTGGT CTGCAATGC CAGTGGCTT GCTGTATCTA AGTCTGCA CCTAGAAGG GAAGTGAACA
 TCCTTTCTT CTAAGAACC AAAACAAT CCACAACCTA GAGTATGAG GTACCCGCA CGACATAGAT TCCAGGAGT GGATCTTCCC CTTCACTTGT

 F2

 701 I K S A L L S T N K A V V S L S N G V S V L T S K V L D L K N Y I
 AGATCAAAAG TGCTCTACTA TCCACAACA AGGCTGTAGT CAGCTTATCA AATGGAGTTA GTGCTTAAAC CAGCAAGTG TTAGACCTCA AAAACTATAT
 TCTAGTTTC ACGAGATGAT AGGTGTTGT TCCGACATCA GTCGAATAGT TTACCTCAAT CACAGAATTG GTCGTTTCAC AATCTGGAGT TTTTGATATA

 F2

 801 D K Q L L P I V N K Q S C S I S N I E T V I E F Q Q K N N R L L E
 AGATAAACA TTGTTACCTA TTGTGAACA GCAAGCTGC AGCATATCAA ATATAGAAG TGTGATAGAG TTCCAACAAA AGAACAACAG ACTACTAGAG
 TCTATTGTT AACAAAGGAT AACACTGTT CGTTTCGAG TCGTATAGT TATATCTTG ACATATCTC AAGTTGTTT TCTTGTGTC TGATGATCTC

 F2

 901 I T R E F S V N A G V T T P V S T Y M L T N S E L L S L I N D M P I
 ATTACAGG AATTTAGT TAATGAGGT GTAATACAC CTGTAAGCAC TACATGTTA ACTAATAGT AATYATGTC AATYATGTC ATTAATCAAT GATATGCCTA
 TAATGGTCCC TTAATCACA ATTACGCCA CATTGATG GACATCTG AATGTACAAT TGATTATCAC TTAATAACAG TAATTAGTTA CTATACGGAT

 F2

 1001 T N D Q K K L M S N N V Q I V R Q Q S Y S I M S I I K E E V L A Y
 TAACAATGA TCAGAAAAG TTAATGTCCA ACAATGTTCA AATAGTTAGA CAGCAAGTT ACTCTATCAT GTCCATAATA AAAGAGGAAG TCTTAGCATA
 ATTTGTTACT AGTCTTTTC AATTACAGGT TGTACAAAGT TTATCAATCT GTCGTTTCAA TGAGATAGTA CAGGTATTAT TTTCTCTTC AGAATCGTAT

 F2

 V V Q L P L Y G V I D T P C W K L H T S P L C T T N T K E G S N I

1101 TGTAGTACAA TTACCACTAT ATGGTGTAT AGATACACCC TGTGGAAC TACACATC CCCTCTATGT ACAACCAACA CAAAAGAAGG GTCCACATC
ACATCATGTT AATGGTGATA TACCACAATA TCTATGTGG ACAACCTTGG ATGTGTGTAG GGGAGATACA TGTTGGTGTGTTTTCTTCC CAGGTGTAG
F2

1201 C L T R T D R G W Y C D N A G S V S F F P Q A E T C K V Q S N R V F
TGTTTAAACA GAACGACAG AGGATGGTAC TGTGACATG CAGGATCAGT ATCTTCTTC CCACAGCTG AACATGTAA AGTTCAATCA AATCGAGTAT
ACAAATTGTT CTTGACTGTC TCCTACCATG ACATGTTAC GTCCTAGTCA TAGAAGAAG GGTGTTCCGAC TTGTACATTT TCAAGTTAGT TTAGCTCAT
F2

1301 C D T M N S L T L P S E I N L C N V D I F N P K Y D C K I M T S K
TTTGTGACAC AATGAACAGT TTAACATTTAC CAAGTGAAT AATCTCTGC AATGTGACA TATCAACCC CAATATCAT TGTAAATTA TGACTTCAA
AAACACTGIG TTACTTGTCA AATGTGAATG GTTCACITTA TTTAGAGAG TTACAGAGG TTAACACTGT ATAAGTTGGG GTTTATACTA ACATTTAAT ACTGAAGTTT
F2

1401 T D V S S V I T S L G A I V S C Y G K T K C T A S N K N R G I I
AACAGATGA AGCAGCTCG TTATCACATC TCTAGGAGCC ATTGTGTCTAT GCTATGGCAA AACTAAATGT ACAGCATCCA ATAAATCG TGAATCAT
TTGTCTACAT TCGTCGAGGC AATAGTGTAG AGATCCTCGG TAACACAGTA CGATACCGTT TTGATTTTACA TGTCGTAGT TATTTTAGC ACCTTAGTAT
F2

1501 K T F S N G C D Y V S N K G M D T V S V G N T L Y Y V N K Q E G K S
AAGCATTTT CTAACGGGAG CGATTATGTA TCAATAAAG GGATGGACAC TGTTCTCTGA GGTAAACAT TATATTATGT AAATAAGCAA GAAGTAA
TTCTGTAAAA GATTGCCAC GCTAATACAT AGTTTATTTC CTTACTGTG ACACAGACAT CCATGTGTA ATATAATACA TTTATTGTT CTTCATTTT
F2

1601 L Y V K G E P I I N F Y D P L V F P S D E F D A S I S Q V N E K I
GTCTCTATGT AAAAGGTGAA CCAATAATAA ATTCTATGA CCTATAGTA TTCCCTCTG ATCAATTTGA TGCATCAATA TCTCAAGTCA ACAGAGAT
CAGAGATACA TTTTCCACTT GGTATTATT TAAAGTACT GGTAAATCAT AAGGGAGAC TACTTAACT ACGTAGTTAT AGATTTCAGT TGCTCTCTA
F2

1701 N Q S L A F I R K S D E L L H N V N A G K S T T N I M I T T I I I
TAACAGAGC CTAGCATTTA TTGTTAAATC CGATGAATTA TTACATAATG TAAATGCTGG TAAATCCACC ACAATATCA TGATAACTAC TATAATTATA
ATTGGTCTCG GATCGTAAAT AAGCATTTAG GCTACTTAAT AATGTATTAC ATTACGACC ATTAGGTGG TGTATTATAGT ACTATTGATG ATATTAAATAT
TM Domain

1801 V I I V I L L S L I A V G L L L Y C K A R S T P V T L S K D Q L S G
GTGATTATAG TAATATTGTT ATCATTAAT GCTGTGGAC TGCTTTATA CTGPAAGGC AGAAGCACAC CAGTCACACT AAGCAAGAT CAACGTGAGT
CACTAATATC ATTATAACAA TAGTAATTA CGACACCTG ACAGAATAT GACATCCGG TCTTCGTG GTCAGTGA TTCGTTTCTA GTTGACTCAC
FMDV 2A

1901 I N N I A F S N N F D L L K L A G D V E S N P G P A R D R S I A L
GTATAAATAA TATGCAATTT AGTAAATTT TTGATCTGCT CAACCTGCA GGGATGTAG AATCAATCC TGGACCGCC CGGACAGGT CCATAGCTCT
CATATTTATT ATACGTAA TCATTGTAA AACTAGCA GTTTGAACGT CCGCTACATC TTAGTTTAGG ACCTGGCGG GCCTGTCCA GGTATCGAGA
Transmembrane domain of WNV E (split)

membrane domain of WNV E (split)

Cytoplasmic Tail

pre E/NS1 signal

NS1

2001 . T F L A V G G V L L F L S V N V H A D T G C A I D I S R Q E L R
 CACGTTTCTC GCAGTTGGAG GAGTTCTGCT CTTCCTCTCC GTGACGTGC ACCTGACAC TGGGTGTGCC ATAGACATCA GCCGCAAGA GCTGAGA
 GTGCAAGAG CGTCAACCTC CTCAGACGA GAAGGAGAG CACTTGACG TGGACTGTG ACCCACAGG TATCTGTAGT CGGCGTTCT CGACTCT

PIV-WNV(Δ C)/RSV-F

1 GATCCTAATA CGACTACTA TAGAGTAGTT CGCTGTGTG AGCTACAAA CTGTAGTAGT TTGTGAGGA TTAACAACA TTAACACAGT GCGAGCTGTT
 CTAGGATTAT GCTGAGTGAT ATCTCATCA GCGACACAC TCGACTGTTT GAATCATCAC AAACACTCTT AATTGTGTT AATTGTGTTA CGCTCGACAA

N-terminus of C

101 TCTTAGCAGC AGATCTCGA TGTCTAAGAA ACCAGAGGG CCGGCAAGA GCCGGGTGT CAATATGCTA AAACGCGAA TGCCCGCGT GTTGTCTTGT
 AGAATCGTGC TTCTAGAGCT ACAGATTCTT TGGTCTCTCC GGGCGTTCT CGGCCGACA GTTATAGAT TTGCGCCTT ACGGGGCGCA CAACAGAAC
 N-terminus of C F signal

NS3 cleavage

F1

201 I G L K Q K K R G G E L L I L K A N A I T T I L T A V T F C F A S G
 ATTGGACTTA AGCAAAAGAA GCGAGGGGC GAGTTGCTAA TCCTCAAGC AAATGCAAT ACCCAATCC TCACGTGAGT CACATTTGT TTGCTTCTG
 TAACCTGAAT TCGTTTCTT CGCTCCCG CTCACGATT AGGAGTTTGG TTACGTGTTA TGGTGTAGG AGTGAGTCA GTGTAACAACA AAACGAGAC

F1

301 Q N I T E E F Y Q S T C S A V S K G Y L S A L R T G W Y T S V I T
 GTCAAAACAT CACTGAAGAA TTTTATCAAT CAACATGCAG TGCAGTTAGC AAAGGCTATC TTAGTGTCTT GAGAAGTGGT TGGTATACCA GTGTATTAAC
 CAGTTTGTGA GTGACTTCTT AAATAGTTA GTTGACGTC ACGTCAATCG TTCCGATAG AATCAGAGA CTCITGACCA ACCATATGTT CACAATATTTG

F1

401 I E L S N I K E N K C N G T D A K V K L I K Q E L D K Y K N A V T
 TATAGAATTA AGTAATATCA AGGAAATAA GTGTAATGGA ACAGATGCTA AGGTAAATTT GATAAACA GAATTAGATA AATATAAAA TGCTGTAACA
 ATATCTTAAT TCATTATAGT TCCTTTTATT CACATTACCT TGCTACGAT TCCATTTTAA CTATTTGTT CTTAATCTAT TTATATTTT ACGACATTTG

F1

501 E L Q L L M Q S T P P T N N R A R R E L P R F M N Y T L N N A K K T
 GAATTGCAGT TGCTCATGCA AAGCACACCA CCAACAAACA ATCGAGCCAG AAGAGACTA CCAAGTTTA TGAATATAT ACTCAACAT GCCAAAAAA
 CTTAACGTCA ACGAGTACGT TTCGTGTGGT GGTGTGTTGT TAGCTCGGTC TTCTCTTGAT GGTCCAAAT ACTTAATATG TGAGTTGTTA CGGTTTTTTT

F2

F1

601 N V T L S K K R K R R F L G F L L G V G S A I A S G V A V S K V L
 CCAATGTAAC ATTAAGCAAG AAAAGGAAA GAAGATTCTT TGGTTTTTG TTAGTTGTTG GATCTGCAAT CGCAGTGGC GTTCTGTAT CTAGGTCTT
 GGTACATTTG TAAATCGTTC TTTTCTTTT TTCTTAAAGA ACCAAAAAC AATCCCAAC CTAGAGTTA GCGGTACCG CAACGACATA GATTCCAGGA

F2

701 H L E G E V N K I K S A L L S T N K A V V S L S N G V S V L T S K
 GCACCTAGAA GGGGAAGTGA ACAAGATCAA AAGTCTCTTA CTATCCCAA ACAAGGTGT AGTCAGTTA TCAATGGAG TTAGTGTCTT AACCAGCAAA
 CGTGGATCTT CCCCTTCACT TGTCTAGTT TACAGAGAT GATAGGTGTT TGTTCGACA TCAGTCAAT AGTTTACCTC AATCAGAA TTGGTCTGTT

F2

V L D L K N Y I D K Q L L P I V N K Q S C S I S N I E T V I E F Q Q

801 GTGTTAGACC TCAAAACTA TATAGATAAA CAATTGTTAC CTATTGTGAA CAAGCAAGC TGCAGCATAT CAATATATAGA AACTGTGATA GAGTTCACAC
CACAATCTGG AGTTTITGAT ATATCTATTT GTTAAACAATG GATACACATT GTTCGTTTCG AGTCGTATA GTTTATATCT TTGACACTAT CTCAGGTTG
F2

901 K N N R L L E I T R E F S V N A G V T T P V S T Y M L T N S E L L
AAGAAGACAA CAGACTACTA GAGATTACCA GGGTAATTAG TGTTAATGCA GGTGTAACCA CAGCTGTAAG CAGTACATG TTAACATAA GTGAATTAT
TTTTCTTGT GTCTGATGAT CTCTAATGGT CCTTAATGCT CCATATGAT GGTGACATTC CTGAATGTAC AATTGATTAT CACTTAATAA
F2

1001 S L I N D M P I T N D Q K K L M S N N V Q I V R Q Q S Y S I M S I
GTCAATTAATC AATGATATGC CTATACAAA TGATCAGAAA AAGTTAATGT CCAACAATGT TCAAAATAGT AGACACAAA GTTACTCTAT CATGTCCATA
CAGTAATTAG TTAATAATAG GATATTGTTT ACTAGTCTTT TTCAATTACA GGTGTTTACA AGTTTATCAA TCTGTGTTT CAATGAGATA GTACAGGTAT
F2

1101 I K E V L A Y V V Q L P L Y G V I D T P C W K L H T S P L C T T N
ATAAAGAGG AGCTTTAGC ATATGTAGTA CAATTACCAC TATATGGTGT TATAGTACA CCCTGTGGA AACTACACAC ATCCCTCTA TGTACAACCA
TATTTCTCC TTCAGAATCG TATACATCAT GTTAATGGTG ATATACCACA ATATCTATGT GGGACAACCT TTGAATGTG TAGGGAGAT ACATGTTGGT
F2

1201 T K E G S N I C L T R T D R G W Y C D N A G S V S F F P Q A E T C
ACACAAAAGA AGGTCCAC ATCTGTTTAA CAAGACTGA CAGAGGATGG TACTGTGACA ATGCAGATC AGTATCTTTC TTCCACACAG CTGAACACATG
TGTTTCTT TCCAGGTTG TAGACAAAT GTTCTTGACT GTCTCTACC ATGACACTGT TACGTCTAG TCATAGAAAG AAGGTGTTT GACTTTGTAC
F2

1301 K V Q S N R V F C D T M N S L T L P S E I N L C N V D I F N P K Y
TAAAGTTCAA TCAATCGAG TATTTGTGA CACAATGAAC AGTTTAACAT TACCAAGTGA AATAATCTC TGCATGTTG ACATATTCAA CCCCAATAT
ATTCAAGTT AGTTAGCTC ATAAACACT GTGTTACTTG TCAATTTGTA ATGTTTCACT TTATTTAGAG ACGTTACAC TGTATAAGT GGGTTTATA
F2

1401 D C K I M T S K T D V S S S V I T S L G A I V S C Y G K T K C T A S
GATGTAAAT TTATGACTTC AAAACAGAT GTAACAGCT CCGTTATCAC ATCTTAGGA GCAATGTGT CATGCTATGG CAAACTAAA TGTACAGCAT
CTAACATTTT AATACTGAAG TTTTGTCTA CATTCGTCGA GGCATAGTG TAGAGATCCT CGGTACACA GTACGATACC GTTTGATTT ACATGCTGTA
F2

1501 N K N R G I I K T F S N G C D Y V S N K G M D T V S V G N T L Y Y
CCAATAAAA TCGTGGATC ATAAAGACAT TTTCTAACGG GTGCGATTAT GTATCAATA AAGGATGGA CACTGTGTCT GTAGTAACA CATTATATTA
GGTTATTTT AGCACCTTAG TATTTCTGTA AAAGATTGCC CACGCTAATA CATAGTTTAT TTCCCTACCT GTGACACAGA CATCCATTGT GTAATATAT
F2

1601 V N K Q E G K S L Y V K G E P I I N F Y D P L V F P S D E F D A S
TGTAATAAG CAAGAAGTA AAAGTCTCTA TGTAAGGT GAACCAATAA TAAATTTCTA TGACCCATTA GTATTTCCCT CTGATGAAT TGTGATCATCA
ACATTTATTC GTTCTTCCAT TTTCAGAGAT ACATTTTCCA CTTGGTTTAT ATTTAAGAT ACTGGGTAAT CATAAGGGA GACTACTTAA ACTACGTAGT
F2

1701 I S Q V N E K I N Q S L A F I R K S D E L L H N V N A G K S T T N I
ATATCTCAAG TCAACGAGAA GATTACCAAG AGCTAGCAT TTATTCGTAA ATCCGATGAA TTATACATA ATGTAATGC TGGTAATCC ACCACAAATA
TATAGAGTTC AGTTGCTCTT CTAATGGTC TCGGATCGTA AATAAGCATT TAGGCTACTT AATAATGTAT TACATTACG ACCATTAGG TGGTGTATT
TM Domain

Cytoplasmic Tail

M I T I I I V I I V I L L S L I A V G L L L Y C K A R S T P V T

1801	TCATGATAAC TACTATAATT ATAGTGATTA TAGTAATATT GTTATCATTA ATTGCTGTTG GACTGCTCTT ATACTGTAAG GCCAGAAGCA CACCAGTCAC AGTACTATTG ATGATATTAA TATCACTAAT ATCAITATAA CAATAGTAAT TAACGACAAC CTGACGAGAA TATGACATTG CCGTCTTCGT GTGGTCAGTG FMDV 2A
1901	----- Cytoplasmic Tail ----- L S K D Q L S G I N N I A F S N N F D L L K L A G D V E S N P G P ACTAAGCAAA GATCAACTGA GTGGTATAA TAATATTGCA TTTAGTAACA ATTTGATCT GCTCAAACTT GCAGGGGATG TAGAATCAAA TCCTGGACCC TGATTCGTTT CTAGTTGACT CACCATATTT ATTATAACGT AAATCATTTG TAAAACTAGA CGAGTTTGAA CGTCCGCTAC ATCTTAGTTT AGGACCTGGG prM
2001	----- C/prM signal ----- G G K T G I A V M I G L I A C V G A V T L S N F Q G K V M M T V N A GGAGGAAGA CCGGTATTGC AGTCATGATT GGCCTGATCG CCTGGTAGG AGCAGTTACC CTCTCTAACT TCCAAGGAA GGTGATGATG ACGGTAAATG CTTCCTTTCT GGCCATAAAG TCAGTACTAA CCGGACTAGC GGACCATCC TCGTCAATGG GAGAGATTGA AGGTTCCCTT CCACTACTAC TGCCATTTCAC
2101	----- T D V T D V I T I P T A A G K N L C I V R A M D V G Y M C D D T I CTACTGACGT CACAGATGTC ATCAGGATTC CAACAGCTGC TGAAGAAGAAC CTATGCAATG TCAGAGCAAT GGATGTGGGA TACATGTGCG ATGATACTAT GATGACTGCA GTGCTACAG TAGTGCTAAG GTTGTGACG ACCTTTCTTG GATACGTTAC AGTCTCGTTA CCTACACCCT ATGTACACGC TACTATGATA prM
2201	----- T Y E C P V L S A G N D P E D I D C W C T K S A V Y V R Y G R C T CACTTATGAA TGCCCAAGTC TGTCGGCTGG TAATGATCCA GAAGACATCG ACTGTTGGTG CACAAAGTCA GCAGTCTACG TCAGGTATGG AAGATGCACC GTGAATACTT ACGGGTCAG ACAGCCGACC ATTACTAGGT CTCTGTAGC TGACAACCCAC GTGTTTCAGT CGTCAGATGC AGTCCATACC TTCTACGTGG prM
2301	----- K T R H S R R S R S L T V Q T H G E S T L A N K K G A W M D S T K AAGACAGCC ACTCAAGAGC CAGTCGGAGG TCACCTGACAG TGCAGACACA CGAGAAAGC ACTCTAGCGA ACAAGAGGG GCGTTGGATG GACAGCACCA TTCTGTGGG TGAGTTCTGC GTCAGCCTCC AGTCACTGC ACCTCTGCTG GCTCTTTCC TGAGATCGCT TGTCTTCCC CCGAACCTAC CTGTGCTGCT prM
2401	----- A T R Y L V K T E S W I L R N P G Y A L V A A V I G W M L G S N T AGGCCACAAG GTATTGTTGTA AAAACAGAAT CATGGATCTT GAGGAACCTT GGATATGCC TGGTGGCAGC CGTCAATGGT TGGATGCTTG GGAGCAACAC TCCGGTGTTC CATAAACCAT TTTTGCTTGA GTACCTAGAA CTCCTTGGA CCTATACGG ACCACCGTCG GCAGTAAACA ACCTAGGAAC CCTCGTTTGT prM
2501	----- M Q R V V F V L L L L V A P A Y S F N C L G M S N R D F L E G V CATGCAGAGA GTTGTGTTTG TCGTGCTATT GCTTTTGGTG GCCCAGCTT ACAGCTTTAA CTGCTTTGGA ATGAGCAACA GAGACTTCTT GGAAGGAGTG GTAGTCTCT CAACACAAC AGCAGGATAA CGAAACCCAC CGGGGTGGA TGTGGAATTT GACGGAACCT TACTCGTTGT CTCTGAAGAA CCTTCTCTAC prM
2601	----- S G A T W V D L V L E G D S C V T I M S K D K P T I D V K M M N M E TCTGGAGCAA CATGGGTGGA TTTGTTCTC GAAGGGACA GCTGGGTGAC TATCATGTCT AAGGACAAGC CTACCATCGA TGTGAAGATG ATGAATATGG AGACCTCGTT GTACCCACCT AAACCAAGAG CTTCGCTGT CGAGCCACTG ATAGTACAGA TTTCTGTTCG GATGTAGCT ACCTTCTAC TACTTATACC E
2701	----- A A N L A E V R S Y C Y L A T V S D L S T K A A C P A M G E A H N AGCGGGCCAA CCTGGCAGAG GTCCGCGATT ATTGCTATT TTGGTACCGTC AGGATCTCT CCACCAAGC TCGCTGCCG GCCATGGGAG AAGCTCACAA TCCGCCGGTT GGACCGTCTC CAGCGGTCNA TAACGATAAA CCGATGGCAG TCCTAGAGA GTGGTTTCG AGCAGCGGC CGGTACCCCTC TTCGAGTGT E

2801 . D K R A D P A F V C R Q G V V D R G W G N G C G L F G K G S I D T
 TGACAAAGT GCTGACCCAG CTTTGTGTG CAGACAGGA GTGTGGACA GGGCTGGG CAAAGGCTGC GGAATATTG GCAAAGGAG CATTGACACA
 ACTGTTTGCA CGACTGGGTC GAAACACAC GTCTGTCTT CACCACCTGT CCGGACCC CTTGCGGAG CCGTGAFAAC CGTTTCTTC GTAACTGTGT
 E
 2901 C A K F A C S T K A I G R T I L K E N I K Y E V A I F V H G P T T V
 TGCGCAAT TTGCTGCTC TACCAAGCA ATAGGAGAA CCAATTTGAA AGAATATC AGTAGAAG TGGCAATTT TGTCCATGGA CCAACTACTG
 ACGCGTTTA ACGGACGAG ATGTTCCGT TATCTCTT TATCTCTT GGTAAACTT TCTCTATAG TTCAATGCTT CCGGTAAAC ACAGTACCT GTTTGATGAC
 E
 3001 . E S H G N Y S T Q V G A T Q A G R F S I T P A A P S Y T L K L G E
 TGGAGTCGA CGAAACTAC TCACACAGG TTGGAGCCAC TCAGGACGG AGATTGACA TCACTCTGTC GCGCTTCA TACACTATA AGCTTGGAGA
 ACCTCAGCT GCCTTGTATG AGGTGTCTC AACCTGGTG AGTCGGTG TCTAAGTCTC TCTAAGTCTC AGTAGGAG CCGCGAAGT ATGTGTGATT TCGAACCTCT
 E
 3101 . Y G E V T V D C E P R S G I D T N A Y Y V M T V G T K T F L V H R
 ATATGGAGG GTGACAGTG ACTGTGAAC ACGGTGAGG ATTGACCA ATGCATCTA CGTATGACT GTTGAACA AGACGTTCTT GGTCCATCTG
 TATACCTCTC CACTGTACC TGACACTGG TGCCAGTCC TAACGTGTG TACGTATGAT GCACTACTGA CAACCTGTT TCTGCAAGAA CCAGTAGCA
 E
 3201 E W F M D L N L P W S S A G S T V W R N R E T L M E F E P H A T K
 GAGTGGTTCA TGGACCTCAA CTTCCCTTG AGCAGTGTG AGTGTGCTG GTGAGGAGT TAATGAGTT TGAAGACCA CAGGCAAGCA
 CTCACCAAGT ACCTGGAGT GAGGGAACC TGTGACGAC CATTGATGACA CACTCTCTG TCTCTGCA ATTACCTCAA ACTCCTGTT GTGGGTGT
 E
 3301 . Q S V I A L G S Q E G A L H Q A L A G A I P V E F S S N T V K L T
 AGCAGTCTGT GATAGCAITG GGTCAACAG AGGAGCTCT GCATCAAGT TTGGCTGAG CCACTCTGT GGAATTTCA AGCACTG TCAAGTTGAC
 TCGTCAGACA CTATCGTAAC CCGAGTGT TCCCTGAGA CGTAGTTGA AACGACCTC GGTAAAGCA CTTTAAAGT TCGTTGTGAC AGTTCAACTG
 E
 3401 . S G H L K C R V K M E K L Q L K G T T Y G V C S K A F K F L G T P
 GTGGGTCTAT TTGAAGTGA GAGTGAAGT GGAATATG CAGTTGAAG GAAACACCTA TGGCTGTGT TCAAGCTT TCAAGTTCT TGGAGTCC
 CAGCCAGTA AACTTCACAT CTCACCTTA CTTTCTTAAC GTCACTTCC CTTGTGTGAT ACCGACACA AGTTTCGAA AGTTCAAGA ACCGTGAGG
 E
 3501 A D T G H G T V V L E L Q Y T G T D G P C K V P I S S V A S L N D L
 GCAGACAG GTGACGGCAC TGTGTGTG GATGTGAGT ACATGGAC GATGGACCT TCAAGTCT CTATCTGTC AGTGGCTTCA TTGAACGACC
 CGTCTGTGTC CAGTGGCGTG ACACCAAC CTTAAGTCA TGTACCGTG CTTACCTGGA AGTTTCAAG GATAGACAG TCACCGAAGT AACTGTCTG
 E
 3601 . T P V G R L V T V N P F V S V A T A N A K V L I E L E P P F G D S
 TAAGCCAGT GGCAGATTG GTCACTGTA ACCCTTTGT TTAGTGGCC ACGGCAAG CTAAGTCTT GATTGATTT GAAACACCT TTGGAGACTC
 ATTGCGGTCA CCGTCTAAC CAGTACAGT TGGGAAACA AGTCAACCG TGGCGTTGTC GATTCAGGA CTAACCTAAC CTTGTGGGA AACCTCTGAG
 E
 3701 . Y I V V G R G E Q Q I N H H W H K S G S S I G K A F T T T L K G A
 ATACATAGT GTGGGACAG GAGAACACA GATCAATCAC CACTGGACA AGTCTGGAAG CAGATTGGC AAGCTTTA CAACACCTT CAAAGGAGCG
 TATGATAC CACCCGTCTC CTCTTGTGT CTAGTTAGT GTGACCTGT TCAGACCTTC GTGTAACCG TTTCGGAAT GTTGTGGGA GTTCTCTCGC
 E
 3801 Q R L A A L G D T A W D F G S V G G V F T S V G K A V H Q V F G G A
 GAGACTAG CCGCTCTAGG AGACAGCT TGGACTTG GATCAGTTG AGGGGTGTC ACCTAGTTG GGAAGCTGT CCATCAAGT TTCGAGGAG
 GTCTCTGATC GCGAGATCC TCTGTGTGA ACCCTGAAC CTAGTCAAC TCCACACAAG TGGAGTCAAC CTTTCGACA GGTAGTTAC AAGCTCTCTC

E

3901
CATTCCGCTC ACTGTTCCGA GGCATGTCCT GGATAACGCA AGGATTGCTG GGGGCTCTCC TGTGTGGAT GGGCATCAAT GCTCGTGACA GGTCCATAGC
GTAAGGCGAG TGACAAGCCT CCGTACAGGA CCTATTGCGT TCCTAACGAC CCCCGAGAGG ACAACACCTA CCCGTAGTTA CGAGCACTGT CCAGGTATCG
NS1

E

4001
TCTCACGTTT CTGCAGTTG GAGGAGTTCT GCTCTTCTC TCCGTGAACG TGCACGCTGA CACTGGGTGT GCCATAGACA TCAGCCGGCA AGAGCTGAGA
AGAGTGCAAA GAGGTC AAC CTCCTCAAGA CGAGAGGAG AGGCACTTGC ACGTGCGACT GTGACCCACA CGGTATCTGT AGTCGCCCGT TCTCGACTCT

Other Embodiments

All publications, patent applications, and patents mentioned in this specification are incorporated herein by reference in their entirety as if each individual publication, patent application, or patent were specifically and individually indicated to be
5 incorporated by reference.

Various modifications and variations of the described viruses, vectors, compositions, and methods of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the
10 invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the fields of medicine, pharmacology, or related fields are intended to be within the scope of the invention. Use of singular forms herein, such as “a” and “the,” does not exclude indication of the corresponding plural form, unless the
15 context indicates to the contrary. Similarly, use of plural terms does not exclude indication of a corresponding singular form. Other embodiments are within the scope of the following claims.

What is claimed is:

1. A replication-deficient pseudoinfectious flavivirus comprising a flavivirus genome comprising (i) one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), and (ii) a sequence encoding one or more heterologous pathogen, cancer, or allergy-related immunogens.
2. The replication-deficient pseudoinfectious flavivirus of claim 1, wherein said one or more deletions or mutations is within capsid (C) sequences of the flavivirus genome.
3. The replication-deficient pseudoinfectious flavivirus of claim 1, wherein said one or more deletions or mutations is within pre-membrane (prM) and/or envelope (E) sequences of the flavivirus genome.
4. The replication-deficient pseudoinfectious flavivirus of claim 1, wherein said one or more deletions or mutations is within capsid (C), pre-membrane (prM), and envelope (E) sequences of the flavivirus genome.
5. The replication-deficient pseudoinfectious flavivirus of claim 1, wherein said one or more deletions or mutations is within non-structural protein 1 (NS1) sequences of the flavivirus genome.
6. The replication-deficient pseudoinfectious flavivirus of any of claims 1-5, wherein said heterologous immunogen is from a pathogen selected from the group consisting of a rabies virus, *Borrelia burgdorferi*, a tick, an influenza virus, a human immunodeficiency virus, a simian immunodeficiency virus, a human papilloma virus, a respiratory syncytial virus, malaria parasite, and *Mycobacterium tuberculosis*.

7. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises a rabies virus G protein immunogen or an immunogenic fragment thereof.

8. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises *Borrelia burgdorferi* OspA immunogen or an immunogenic fragment thereof.

9. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises a tick saliva protein selected from the group consisting of 64TRP, Isac, and Salp20, or an immunogenic fragment thereof.

10. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises an influenza virus M2, hemagglutinin (HA), or neuraminidase (NA) epitope, or an immunogenic fragment thereof.

11. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises a codon-optimized HIV gag, tat/nef, or gp120 protein, or an immunogenic fragment thereof.

12. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises an HPV16 or HPV18 capsid protein L1 or L2, or an immunogenic fragment thereof.

13. The replication-deficient pseudoinfectious flavivirus of claim 6, wherein said heterologous immunogen comprises a respiratory syncytial virus F or G glycoprotein.

14. The replication-deficient pseudoinfectious flavivirus of any of claims 1-13, wherein said flavivirus genome comprises sequences encoding a pre-membrane (prM) and/or envelope (E) protein.

15. The replication-deficient pseudoinfectious flavivirus of any of claims 1-14, wherein the flavivirus genome is selected from that of yellow fever virus, West Nile virus, tick-borne encephalitis virus, Langat virus, Japanese encephalitis virus, dengue virus, and St. Louis encephalitis virus sequences, and chimeras thereof.

16. The replication-deficient pseudoinfectious flavivirus of claim 15, wherein said chimera comprises pre-membrane (prM) and envelope (E) sequences of a first flavivirus, and capsid (C) and non-structural sequences of a second, different flavivirus.

17. The replication-deficient pseudoinfectious flavivirus of claim 16, wherein said first flavivirus is a tick-borne encephalitis virus or a Langat virus.

18. The replication-deficient pseudoinfectious flavivirus of claim 16 or 17, wherein said second, different flavivirus is a yellow fever virus or a West Nile virus or Langat virus.

19. The replication-deficient pseudoinfectious flavivirus of any of claims 1-18, wherein said genome is packaged in a particle comprising pre-membrane (prM) and envelope (E) sequences from a flavivirus that is the same or different from that of the genome.

20. The replication-deficient pseudoinfectious flavivirus of any of claims 1-19, wherein sequences encoding said heterologous immunogen are inserted in the place of or in combination with the one or more deletions or mutations of the one or more proteins.

21. The replication-deficient pseudoinfectious flavivirus of any of claims 1-20, wherein sequences encoding said heterologous immunogen are inserted in the flavivirus genome within sequences encoding the envelope (E) protein, within sequences encoding the non-structural 1 (NS1) protein, within sequences encoding the pre-membrane (prM) protein, intergenically between sequences encoding the envelope (E) protein and non-

structural protein 1 (NS1), intergenically between non-structural protein 2B (NS2B) and non-structural protein 3 (NS3), or as a bicistronic insertion in the 3' untranslated region of the flavivirus genome.

22. A composition comprising a first replication-deficient pseudoinfectious flavivirus of any of claims 1-21 and a second, different replication-deficient pseudoinfectious flavivirus comprising a genome comprising one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein the one or more proteins encoded by the sequences in which the one or more deletion(s) or mutation(s) occur in the second, different replication-deficient pseudoinfectious flavivirus are different from the one or more proteins encoded by the sequences in which the one or more deletion(s) or mutation(s) occur in the first replication-deficient pseudoinfectious flavivirus.

23. A method of inducing an immune response to an immunogen in a subject, the method comprising administering to the subject one or more replication-deficient pseudoinfectious flaviviruses of any of claims 1-21 and/or a composition of claim 22 to the subject.

24. The method of claim 23, wherein the subject is at risk of but does not have an infection by said pathogen or a disease or condition associated with said cancer or allergy-related immunogen.

25. The method of claim 23, wherein the subject has an infection by said pathogen or a disease or condition associated with said cancer or allergy-related immunogen.

26. The method of any of claims 23-25, wherein the immunogen is from a pathogen selected from the group consisting of a rabies virus, *Borrelia burgdorferi*, a tick, an influenza virus, a human immunodeficiency virus, a simian immunodeficiency virus, a human papilloma virus, a respiratory syncytial virus, malaria parasite, and *Mycobacterium tuberculosis*.

27. The method of any of claims 23-26, wherein the method is for inducing an immune response against a protein encoded by the flavivirus genome, in addition to the source of the immunogen.

28. The method of claim 27, wherein the subject is at risk of but does not have an infection by the flavivirus corresponding to the genome of the pseudoinfectious flavivirus, which comprises sequences encoding a flavivirus pre-membrane and/or envelope protein.

29. The method of claim 27, wherein the subject has an infection by the flavivirus corresponding to the genome of the pseudoinfectious flavivirus which comprises sequences encoding a flavivirus pre-membrane and/or envelope protein.

30. A live, attenuated chimeric flavivirus comprising a yellow fever virus in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis virus or a Langat virus, and the signal sequence between the capsid and pre-membrane proteins of the chimeric flavivirus comprises a hybrid of yellow fever virus and tick-borne encephalitis or Langat virus capsid/pre-membrane signal sequences, or a variant thereof.

31. The live, attenuated chimeric flavivirus of claim 30, wherein the capsid/pre-membrane signal sequence of the chimeric flavivirus comprises yellow fever virus

sequences in the amino terminal region and tick-borne encephalitis or Langat virus sequences in the carboxy terminal region.

32. A live, attenuated chimeric flavivirus comprising a West Nile virus in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis or a Langat virus, and the signal sequence between the capsid and pre-membrane proteins of the chimeric flavivirus comprises a tick-borne encephalitis or a Langat virus capsid/pre-membrane signal sequence, or a variant thereof.

33. A pharmaceutical composition comprising a pseudoinfectious flavivirus of any of claims 1-21, the composition of claim 22, or the live, attenuated flavivirus of any of claims 30-32 and a pharmaceutically acceptable carrier or diluent.

34. The pharmaceutical composition of claim 33, further comprising an adjuvant.

35. A replication-deficient pseudoinfectious flavivirus comprising a flavivirus genome comprising one or more deletions or mutations in nucleotide sequences encoding non-structural protein 1 (NS1), non-structural protein 3 (NS3), or non-structural protein 5 (NS5).

36. A nucleic acid molecule corresponding to the genome of a pseudoinfectious flavivirus of any of claims 1-21 or 35, or the genome the live, attenuated flavivirus of any of claims 30-32.

37. A method of making a replication-deficient pseudoinfectious flavivirus of any of claims 1-21 or 35, the method comprising introducing a nucleic acid molecule of claim 36 into a cell that expresses the protein corresponding to any sequences deleted from the flavivirus genome of the replication-deficient pseudoinfectious flavivirus.

38. The method of claim 37, wherein the protein is expressed in the cell from the genome of a second, different, replication-deficient pseudoinfectious flavivirus.

39. The method of claim 37, wherein the protein is expressed from a replicon.

40. The method of claim 39, wherein the replicon is an alphavirus replicon.

41. The method of claim 40, wherein the alphavirus is a Venezuelan Equine Encephalitis virus.

42. A composition comprising two or more replication-deficient pseudoinfectious flaviviruses, wherein two of the replication-deficient pseudoinfectious flaviviruses are selected from the groups consisting of:

(a) a replication-deficient pseudoinfectious flavivirus comprising a genome comprising Japanese encephalitis virus sequences, and a replication-deficient pseudoinfectious flavivirus comprising a genome comprising dengue virus sequences;

(b) a replication-deficient pseudoinfectious flavivirus comprising a genome comprising yellow fever virus sequences, and a replication-deficient pseudoinfectious flavivirus comprising a genome comprising dengue virus sequences; and

(c) a replication-deficient pseudoinfectious flavivirus comprising a genome comprising tick-borne encephalitis or Langat virus sequences and an inserted sequence encoding a *Borrelia burgdorferi* immunogen, and a replication-deficient pseudoinfectious flavivirus comprising a genome comprising tick-borne encephalitis or Langat virus sequences and an inserted sequence encoding a tick saliva protein immunogen, or a replication-deficient pseudoinfectious flavivirus comprising a genome comprising tick-borne encephalitis or Langat virus sequences and inserted sequences encoding a *Borrelia burgdorferi* immunogen and a tick saliva protein immunogen.

43. A pharmaceutical composition comprising the live, attenuated chimeric flavivirus of any of claims 30-32.

44. A method of inducing an immune response to tick-borne encephalitis virus or Langat virus in a subject, the method comprising administering to the subject the pharmaceutical composition of claim 43.

45. The method of claim 44, wherein the subject does not have but is at risk of developing infection by tick-borne encephalitis virus or Langat virus.

46. The method of claim 44, wherein the subject is infected with tick-borne encephalitis virus or Langat virus.

47. A replication-deficient pseudoinfectious flavivirus comprising a flavivirus genome comprising one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein the flavivirus genome comprises yellow fever virus sequences in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis virus or a Langat virus, and sequences encoding the signal sequence between the capsid and pre-membrane proteins of the flavivirus genome comprise a hybrid of sequences encoding yellow fever virus and tick-borne encephalitis or Langat virus capsid/pre-membrane signal sequences, or a variant thereof.

48. The replication-deficient pseudoinfectious flavivirus of claim 47, wherein the sequences encoding the capsid/pre-membrane signal sequence of the flavivirus genome comprise yellow fever virus sequences in the 5' region and tick-borne encephalitis or Langat virus sequences in the 3' region.

49. A replication-deficient pseudoinfectious flavivirus comprising a flavivirus genome comprising one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane

(prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein the flavivirus genome comprises West Nile virus sequences in which sequences encoding pre-membrane and envelope proteins are replaced with sequences encoding pre-membrane and envelope proteins of a tick-borne encephalitis or a Langat virus, and the sequences encoding the signal sequence between the capsid and pre-membrane proteins of the flavivirus genome comprise sequences encoding a tick-borne encephalitis or a Langat virus capsid/pre-membrane signal sequence, or a variant thereof.

50. A replication-deficient pseudoinfectious flavivirus comprising a flavivirus genome comprising one or more deletions or mutations in nucleotide sequences encoding one or more proteins selected from the group consisting of capsid (C), pre-membrane (prM), envelope (E), non-structural protein 1 (NS1), non-structural protein 3 (NS3), and non-structural protein 5 (NS5), wherein any capsid (C) and non-structural (NS) proteins in said flavivirus genome are from Langat virus and any pre-membrane (prM) and envelope (E) proteins are from a tick-borne encephalitis virus.