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Patentanmeldung Nr.

Patent application No.

Demande de brevet n°

16161068.8 / EP16161068

The organization code and number of your priority application, to be used for filing abroad under the Paris Convention, is EP16161068.

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.



V. Joseph

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INACTIVATED ZIKA VIRUS VACCINE

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INACTIVATED ZIKA VIRUS VACCINE

FIELD OF INVENTION

The disclosure relates to Zika virus vaccines and compositions and methods for
5 producing said vaccines and administering the vaccines to subjects for the generation of an
anti-Zika virus immune response.

BACKGROUND OF THE INVENTION

In 2007, Zika virus was detected for the first time outside of the endemic regions of
10 Asia and Africa since its discovery in a Rhesus monkey in Uganda in 1947. Since then, the
virus has caused a large epidemic in French Polynesia, spreading through islands in the
Pacific and into South and Central America by 2015 (WHO “Zika Situation Report” February
5, 2016). Evidence suggests that in addition to being transmitted by *Aedes* species
mosquitos, other vectors may exist, and the virus may be transmitted by blood transfusion,
15 transplacentally, and through sexual transmission (WHO Zika Virus Fact Sheet, Feb. 2016).
Though the symptoms of Zika virus infection include mild fever, rash, and conjunctivitis,
there is a likely correlation between infection and neurological disorders, including Guillain-
Barré syndrome and microcephaly in fetuses/neonates subsequent to infection during
pregnancy. There is currently no specific treatment or vaccine for Zika virus and the only
20 preventative measurements involve control of the mosquito vector. Zika virus presents a
substantial public health threat due to the wide circulation of the *Aedes* mosquito, multiple
routes of transmission, and potentially severe neurological effects of infection.

SUMMARY OF THE INVENTION

25 Disclosed herein are Zika virus vaccines and compositions comprising an inactivated
Zika virus, and related methods of producing said vaccines and compositions. Also provided
are methods of administering the Zika virus vaccines for the prevention of Zika virus
infection and/or for the production of an anti-Zika virus immune response in subjects, for
30 example subjects at risk of being exposed to Zika virus. In particular, the invention is directed
to a Zika virus vaccine comprising an optimally inactivated Zika virus particle, wherein the

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Zika virus particle in an appropriate dose is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability, preferably an 80% probability.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing, alignments were performed with the multi alignment package Jalview (Waterhouse et al., 2009, Bioinformatics 25 (9) 1189-1191). In the drawings:

Figure 1: Average distance tree (by % identity, nt), complete genomes.

Figure 2: Neighbor joining tree (by % identity, nt), complete genomes.

Figure 3: Pairwise alignment-Jalview (% identity, nt), complete genomes.

Figure 4: Average distance tree (by % identity, aa), E-protein.

Figure 5: Neighbor joining tree (by % identity, aa), E-protein.

Figure 6: Pairwise alignment-Jalview (% identity, aa), E-protein.

Figure 7: Alignment (blue shading: % identity, aa), E-protein.

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are Zika virus vaccines and compositions comprising an inactivated Zika virus, and related methods of producing said vaccines and compositions. Also provided are methods of administering the Zika virus vaccines for the prevention of Zika virus infection and/or for the production of an anti-Zika virus immune response in subjects, for example subjects at risk of being exposed to Zika virus.

Zika virus is a flavivirus closely related to Dengue virus and is similarly transmitted by the *Aedes* species mosquito, although other arthropod vectors for Zika virus are possible. Since it was first isolated from a Rhesus monkey in the Zika forest of Uganda in 1947, there were very few reported incidents of human infection, especially outside of the endemic
5 regions of Africa and Asia until a large outbreak in French Polynesia in 2007 (Haddow *et al. PLoS Neglected Tropical Diseases* (2012) 6(2), Malone *et al. PLoS Neglected Tropical Diseases* (2016) 10(3),). The virus has since spread through islands of the Pacific, including Oceania, and into South and Central America (WHO “Zika Situation Report” February 5, 2016).

10 In addition to being spread by the bite of an infected mosquito, evidence also suggests transmission may occur between individuals, such as from the blood of an infected individual, *in utero*/transplacental transmission from an infected mother to the fetus, sexual transmission between sexual partners, and possibly by other local transmission routes. There is a possible association between Zika virus infection during pregnancy and microcephaly in
15 the fetus/neonate. Microcephaly is a rare condition in which a baby’s head circumference is significantly less than expected based on the average for their age, sex, and ethnicity. This is a result of the brain failing to undergo proper embryonic development, and in 90% of cases is associated with mental retardation (Rocha *et al. (2016) Bull World Health Organ* 8 Feb. 2016).

20 There is a probable association between individuals having had a prior Zika virus infection and the incidence of Guillain-Barré syndrome, a neurological disorder in which the individual’s immune system destroys the myelin sheath surrounding axons of the peripheral nervous system (WHO “Zika Situation Report” February 5, 2016).

No specific treatments or vaccines for Zika virus currently exist, and the only
25 measures at this time to prevent infection are through vector control and avoiding travel to regions experiencing outbreaks.

Described herein are Zika virus vaccines and compositions comprising inactivated Zika virus that provide a safe method for generating an immune response to Zika virus, including virus-neutralizing antibodies, that may help prevent against Zika virus infection.

30 Any strain of Zika virus may be used in the methods and compositions described herein. In some embodiments, the Zika virus is an isolate from an infected subject during a Zika virus outbreak. In some embodiments, the Zika virus is a strain isolated from Africa or

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from the African virus lineage. In some embodiments, the Zika virus is a strain isolated from Asia or from the Asian lineage (includes also strains from French Polynesia). In some embodiments, the Zika virus is a strain isolated from the Americas (South America, Central America, or North America), such as a Suriname Zika virus strain. In some embodiments, the Zika virus has a RNA genome corresponding (but not limited) to the DNA sequence provided by GenBank Accession No. AY632535.2, KU321639.1, KU497555.1, KU501215.1, KU509998.1, KU527068.1, KU681081.3, KU681082.3, KU707826.1, KU744693.1, or LC002520.1.

Provided below are examples of nucleic acid sequences of the genomes of Zika viruses that may be used in the methods, compositions, and/or vaccines described herein.

AY632535.2 NC_012532.1 Zika virus strain MR 766, Uganda, complete genome (SEQ ID NO: 1)

AGTTGTTGATCTGTGTGAGTCAGACTGCGACAGTTCGAGTCTGAAGCGAGAGCTAACAAACAGTATCAACAGGTTT
AATTTGGATTTGGAAACGAGAGTTTCTGGTCATGAAAAACCCCAAAGAAGAAATCCGGAGGATCCGGATTGTCAA
TATGCTAAAACCGGAGTAGCCCGTGTAAACCCCTTGGGAGGTTTGAAGAGGTTGCCAGCCGGACTTCTGCTGGG
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CATGTTGTGACGCCACCATGAGTTATGAGTGCCCTATGCTGGATGAGGGAGTGGAACAGATGATGTCGATTGCTG
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 CCACCACGCTGGCCGCCAGGCACAGATCGCCGAACCTCGGCGGCCGCTGTGGGGAAATCCATGGTTTCT

KU321639.1 Zika virus strain ZikaSPH2015, Brazil, complete genome (SEQ ID NO: 2)

55 GTTGTTACTGTTGCTGACTCAGACTGCGACAGTTCGAGTTTGAAGCGAAAGCTAGCAACAGTATCAACAGGTTTT
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ATGCTAAAACGCGGAGTAGCCCGTGTGAGCCCCTTTGGGGGCTTGAAGAGGCTGCCAGCCGGACTTCTGCTGGGT
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KU497555.1 Zika virus isolate Brazil-ZKV2015, Brazil, complete genome (SEQ ID NO: 3)

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KU509998.1 Zika virus strain Haiti/1225/2014, Haiti, complete genome (SEQ ID NO: 5)

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KU527068.1 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal, complete

5 genome (SEQ ID NO: 6)

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KU681081.3 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14, Thailand,

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15

KU681082.3 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740, Philippines,
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KU744693.1 Zika virus isolate VE_Ganxian, China, complete genome (SEQ ID NO: 10)

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LC002520.1 Zika virus genomic RNA, strain: MR766-NIID, Uganda, complete genome

55 (SEQ ID NO: 11)

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 CGGAGGGTTTGGCAAGGCAGACATTGAGATGGCTGGACCATGCTGTCAGTAGGCTTGCTAATTGTCAGCTATGT

GGTCTCGGGAAAGAGTGTGGACATGTACATTGAAAGAGCAGGTGACATCACATGGGAAAAGGACGCGGAAGTCAC
 TGGAAACAGTCTCGGCTTGACGTGGCACTGGATGAGAGTGGTGATTTCTCCTTGGTAGAGGAAGATGGTCCACC
 CATGAGAGAGATCATACTTAAGGTGGTCCTGATGGCCATCTGTGGCATGAACCCAATAGCTATACCTTTTGCTGC
 5 AGGAGCGTGGTATGTGTATGTGAAGACTGGGAAAAGGAGTGGCGCCCTCTGGGACGTGCCTGCTCCCAAAGAAGT
 GAAGAAAGGAGAGACCACAGATGGAGTGTACAGAGTATGACTCGCAGACTGCTAGGTTCAACACAGTTGGAGT
 GGGAGTCATGCAAGAGGGAGTCTTCCACACCATGTGGCACGTTACAAAAGGAGCCGCACTGAGGAGCGGTGAGGG
 AAGACTTGATCCATACTGGGGGATGTCAAGCAGGACTTGGTGTCTACTGTGGGCCTTGGAAGTTGGATGCAGC
 TTGGGATGGACTCAGCGAGGTACAGCTTTTGGCCGTACCTCCCGGAGAGAGGGCCAGAAACATTACAGACCTTGCC
 10 TGGAAATATTCAAGACAAAGGACGGGGACATCGGAGCAGTTGCTCTGGACTACCCTGCAGGGACCTCAGGATCTCC
 GATCCTAGACAAAATGTGGAAGAGTGATAGGACTCTATGGCAATGGGGTTGTGATCAAGAATGGAAGCTATGTTAG
 TGCTATAACCCAGGGAAAGAGGGAGGAGAGACTCCGGTTGAATGTTTCGAACCCCTCGATGCTGAAGAAGAAGCA
 GCTAACTGTCTTGGATCTGCATCCAGGACCGGAAAAACAGGAGAGTTCTTCTGAAATAGTCCGTGAAGCCAT
 AAAAAAGAGACTCCGGACAGTGATCTTGGCACCACATAGGGTTGTCTGCTGCTGAGATGGAGAGCGCTTGAGAGG
 ACTTCCGGTGCCTTACATGACAACAGCAGTCAACGTCACCCATTCTGGGACAGAAATCGTTGATTGTATGTGCCA
 15 TGCCACTTTCACTTCACGCTTACTACAACCCATCAGAGTCCCTAATTACAATCTCTACATCATGGATGAAGCCCA
 CTTTACAGACCCCTCAAGTATAGCTGCAAGAGGATATATATCAACAAGGGTTGAAATGGGCGAGGCGGCTGCCAT
 TTTTATGACTGCCACACCACAGGAACCCGTGATGCGTTTCTGACTCTAACTCACCATCATGGACACAGAAGT
 GGAAGTCCCAGAGAGAGCCTGGAGCTCAGGCTTTGATTGGGTGACAGACCAATTCTGGGAAAACAGTTTGGTTCTGT
 TCCAAGCGTGAGAAACGGAAATGAAATCGCAGCCTGTCTGACAAAGGCTGGAAAGCGGGTCATACAGCTCAGCAG
 20 GAAGACTTTTGAGACAGAAATTTAGAAAACAAAAATCAAGAGTGGGACTTGTCTATAACAACATGACATCTCAGA
 GATGGGCGCCAACCTCAAGGCTGACCGGGTCATAGACTCTAGGAGATGCCTAAAACAGTCATACTTGATGGTGA
 GAGAGTCATCTTGGCTGGGCCCATGCCTGTACGCATGCTAGTGTCTGCTCAGAGGAGAGGACGTATAGGCAGGAA
 CCCTAACAAACCTGGAGATGAGTACATGTATGGAGGTGGGTGTGTCAGAGACTGATGAAGGCCATGCACACTGGCT
 TGAAGCAAGAAATGCTTCTTGACAACATCTACCTCCAGGATGGCCTCATAGCCTCGCTCTATCGGCCTGAGGCCGA
 25 TAAGGTAGCCGCCATTGAGGGAGAGTTTAAAGCTGAGGACAGAGCAAAGGAAGACCTTCGTGGAACATCATGAAGAG
 AGGAGACCTTCCCGTCTGGCTAGCCTATCAGGTTGCATCTGCCGGAATAACTTACACAGACAGAAGATGGTGCTT
 TGATGGCACAAACCAACACCATAATGGAAGACAGCGTACCAGCAGAGGTGTGGACAAAGTATGGAGAGAAGAG
 AGTGCTCAAACCGAGATGGATGGATGCTAGGGTCTGTTTCAAGCATGCGGCCCTGAAGTCTGTTCAAAGAATTCGC
 CGCTGGA AAAAGAGGAGCGGCTTTGGGAGTAATGGAGGCCCTGGGAACACTGCCAGGACACATGACAGAGAGGTT
 30 TCAGGAAGCCATTGACAACCTCGCCGTGCTCATGCGAGCAGAGACTGGAAGCAGGCCTTATAAGGCAGCGGCAGC
 CCAACTGCCGGAGACCCTAGAGACCATTATGCTCTTAGGTTTGTCTGGGAACAGTTTCACTGGGGATCTTCTTCGT
 CTTGATGCGGAATAAGGGCATCGGGAAGATGGGCTTTGGAATGGTAACCCCTGGGGCCAGTGCATGGCTCATGTG
 GCTTTCCGAAATTGAACCAGCCAGAATTCATGTGTCTCTATTGTTGTGTTTTATTACTGGTGGTGCTCATACC
 CGAGTGCACAAAGCAAGATCTCCCAAGATAACCAATGGCAATTATCATCATGGTGGCAGTGGGCCCTTCTAGG
 35 TTTGATAACTGCAACGAACCTTGGATGGCTGGAAAGACAAAAATGACATAGCTCATCTAATGGGAAGGAGAGA
 AGAAGGAGCAACCATGGGATTTCAATGGACATTGATCTGCGGCCAGCCTCCGCCTGGGCTATCTATGCCGCATT
 GACAACCTCTCATACCCAGCTGTCCAACATGCGGTAACCACTTCATACAACAACCTACTCCTTAATGGCGATGGC
 CACACAAGCTGGAGTGCTGTTTGGCATGGGCAAAGGGATGCCATTTTATGATGGGACCTTGGAGTCCCGCTGCT
 AATGATGGGTTGCTATTCACAATTAACACCCCTGACTCTGATAGTAGCTATCATTCTGCTTGTGGCGCACTACAT
 40 GTACTTGATCCCAGGCCTACAAGCGGCAGCAGCGCTGTGCCCAGAAAAGGACAGCAGCTGGCATCATGAAGAA
 TCCCGTTGTGGATGGAATAGTGGTAACCTGACATTGACACAATGACAATAGACCCCAAGGTGGAGAAGAAGATGGG
 ACAAGTGTTACTCATAGCAGTAGCCATCTCCAGTGCTGTGCTGCTGCGGACCGCCTGGGGATGGGGGGAGGCTGG
 AGCTCTGATCACAGCAGCGACCTCCACCTTGTGGGAAGGCTCTCCAAACAAATACTGGAACCTCTACAGCCAC
 CTCACCTGTGCAACATCTTACAGAGGAAGCTATCTGGCAGGAGCTTCCCTTATCTATACAGTGACGAGAAACGCTGG
 45 CCTGGTTAAGAGACGTGGAGGTGGGACGGGAGAGACTCTGGGAGAGAAGTGGAAAGCTCGTCTGAATCAGATGTC
 GGCCCTGGAGTTCTACTCTTATAAAAAGTCAGGTATCACTGAAGTGTGTAGAGAGGAGGCTCGCCGTGCCCTCAA
 GGATGGAGTGGCCACAGGAGGACATGCCGTATCCCGGGGAAGTGCAAAGCTCAGATGGTTGGTGGAGAGAGGATA
 TCTGCAGCCCTATGGGAAGGTTGTTGACCTCGGATGTGCGAGAGGGGGCTGGAGCTATTATGCCCGCCACCATCCG
 CAAAGTGCAAGGAGGTGAGAGGATACACAAGAGGAGGTTCCCGGTCATGAAGAACCATGCTGGTGCACCAAGCTATGG
 50 GTGGAACATAGTTCGTCTCAAGAGTGGAGTGGACGCTTCCACATGGCGGCTGAGCCGTGTGACACTCTGCTGTG
 TGACATAGGTGAGTCATCATCTAGTCCTGAAGTGGAAAGAGACGAACACTCAGAGTGTCTCTATGGTGGGGGA
 CTGGCTTGAAAAAGACCAGGGGCCCTCTGTATAAAGGTGCTGTGCCATACACCAGCACTATGATGGAAACCAT
 GGAGCGACTGCAACGTAGGCATGGGGGAGGATTAGTCAGAGTGCCATTGTCTCGCAACTCCACACATGAGATGTA
 CTGGGTCTCTGGGGCAAAGAGCAACATCATAAAAAGTGTGTCCACCACAAGTCAGCTCCTCCTGGGACGCATGGA
 55 TGGCCCCAGGAGGCCAGTGAATATGAGGAGGATGTGAACCTCGGCTCGGGTACACGAGCTGTGGCAAGCTGTGC
 TGAGGCTCCTAACATGAAAATCATCGGCAGGCGCATGTGAGAGAATCCGCAATGAACATGCAAGAAATGGTTTCT
 TGATGAAAACCCACATACAGGACATGGGCCTACCATGGGAGCTACGAAGCCCCACGCAAGGATCAGCGTCTTC
 CCTCGTGAACGGGGTTGTTAGACTCCTGTCAAAGCCTTGGGACGTGGTACTGGAGTTACAGGAATAGCCATGAC

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TGACACCACACCATACGGCCAACAAAGAGTCTTCAAAGAAAAAGTGGACACCAGGGTGCCAGATCCCCAAGAAGG
CACTCGCCAGGTAATGAACATAGTCTCTTCCTGGCTGTGGAAGGAGCTGGGGAAACGCAAGCGCCACGCGTCTG
CACCAAAGAAGAGTTTATCAACAAGGTGCGCAGCAATGCAGCACTGGGAGCAATATTTGAAGAGGAAAAAGAATG
5 GAAGACGGCTGTGGAAGCTGTGAATGATCCAAGTTTTGGGCCCTAGTGGATAGGGAGAGAGAACACCACCTGAG
AGGAGAGTGTACAGCTGTGTGTACAACATGATGGGAAAAAGAGAAAAGCAAGGAGAGTTTCGGGAAAGCAAA
AGGTAGCCGCGCCATCTGGTACATGTGGTTGGGAGCCAGATTCTTGGAGTTTGAAGCCCTTGATTCTTGAACGA
GGACCATTGGATGGGAAGAGAAAACCTCAGGAGGTGGAGTGAAGGGTTAGGATTGCAAAGACTTGGATACATTCT
AGAAGAAATGAATCGGGCACCAGGAGGAAAGATGTACGCAGATGACACTGCTGGCTGGGACACCCGCATTAGTAA
10 GTTTGATCTGGAGAATGAAGCTCTGATTACCAACCAATGGAGGAAGGGCAGAGAACTCTGGCGTTGGCCGTGAT
TAAATACACATACCAAAACAAAGTGGTGAAGGTTCTCAGACCAGCTGAAGGAGGAAAAACAGTTATGGACATCAT
TTCAAGACAAGACCAGAGAGGGAGTGGACAAGTTGTCACTTATGCTCTCAACACATTCACCAACTTGGTGGTGCA
GCTTATCCGGAACATGGAAGCTGAGGAAGTGTAGAGATGCAAGACTTATGTTGTTGAGGAAGCCAGAGAAAAGT
GACCATATGGTTGCAGAGCAATGGATGGGATAGACTCAAACGAATGGCGGTGAGTGGAGATGACTGCGTTGTGAA
GCCAATCGATGATAGGTTTGCACATGCCCTCAGGTTCTTGAATGACATGGGAAAAGTTAGGAAAGACACACAGGA
15 GTGGAACCCCTCGACTGGATGGAGCAATTGGGAAGAAGTCCCGTTCTGCTCCCACTTCAACAAGCTGTACCT
CAAGGATGGGAGATCCATTGTGGTCCCTTGCCGCCACCAAGATGAACATGATGGCCGAGCTCGCGTCTCACCAGG
GGCAGGATGGAGCATCCGGGAGACTGCCGTCTTGCAAAATCATATGCGCAGATGTGGCAGCTCCTTTATTTCCA
CAGAAGAGACCTTCGACTGATGGCTAATGCCATTGCTCGGCTGTGCCAGTTGACTGGGTACCAACTGGGAGAAC
CACCTGGTCAATCCATGGAAAGGGAGAATGGATGACCCTGAGGACATGCTCATGGTGTGGAAATAGAGTGTGGAT
20 TGAGGAGAACGACATATGGAGGACAAGACTCCTGTAAACAAAATGGACAGACATTCCTATCTAGGAAAAAGGGA
GGACTTATGGTGTGGATCCCTTATAGGGCACAGACCCCGCACCCTTGGGCTGAAAACATCAAAGACACAGTCAA
CATGGTGCAGGATCATAGGTGATGAAGAAAAGTACATGGACTATCTATCCACCAAGTCCGCTACTTGGGTGA
GGAAGGGTCCACACCCGGAGTGTGTAAGCACCAATTTAGTGTGTGTCAGGCCTGCTAGTCAGCCACAGTTTGGG
GAAAGCTGTGAGCCTGTAACCCCCCAGGAGAAGCTGGGAAACCAAGCTCATAGTCAGGCCGAGAACGCCATGG
25 CACGGAAGAAGCCATGCTGCCTGTGAGCCCCCTCAGAGGACACTGAGTCAAAAAACCCACGCGCTTGAAGCGCA
GGATGGGAAAAGAAGGTGGCGACCTTCCCCACCCTTCAATCTGGGGCCTGAACTGGAGACTAGCTGTGAATCTCC
AGCAGAGGGACTAGTGGTTAGAGGAGACCCCGGAAAACGCAAAACAGCATATTGACGCTGGGAAAGACCAGAG
ACTCCATGAGTTTCCACCACGCTGGCCGCCAGGCACAGATCGCCGAACAGCGGCGCGGTGTGGGGAAATCCAT
GGTTTCT
30

In some embodiments, the Zika virus has a RNA genome corresponding to the DNA
sequence provided by the nucleic acid sequence of any one of SEQ ID NOs: 1-11. In some
embodiments, the Zika virus has a variant genome that is at least 88%, 89%, 90%, 91%, 92%.
93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% identical to
35 any one of SEQ ID NOs: 1-11.

Provided below are amino acid sequences of the example E proteins of Zika viruses
that may be used in the methods, compositions, and/or vaccines described herein.

40 ArD157995.AHL43503.1.-/291-794 polyprotein [Zika virus]. ArD157995.AHL43503.1.-
polyprotein [Zika virus]. tr|W8QFH2|W8QFH2_9FLAV Polyprotein [Zika virus] (SEQ ID
NO: 12)
ISCIGVSNRDLVEGMSGGTWVDVVLHGGCVTEMAQDKPTVDIELVTMTVSNMAEVRSYCYEASLSDMASASRCP
45 TQGEPSLDKQSDTQSVCKRTLGDGRWGNGCGIFGKGSVLVTCSEKFTCCCKMPGKSIQOPENLEYRIMLPVHGSQHS
MIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLLTMNNKHWLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSGEGAVHTALAGALEAEMDGAKEGRLFGHLKCRCLKMDKLRL
KGVSYSLCTAAFTFTKVPAAETLHGTVTVEVQSAGTDGPCKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNLSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLG LNTKNGSISLTCLALGGVMIFLSTAVSA

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- MR_766.ABI54475.1.Uganda/291-788 polyprotein [Zika virus].
MR_766.ABI54475.1.Uganda polyprotein [Zika virus]. tr|C8XPB1|C8XPB1_9FLAV
5 Genome polyprotein [Zika virus] (SEQ ID NO: 13)
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPWHAGADT
GTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRFSGHLKCRKMDKRLKGVSY
10 LCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMMLELDP
FGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGIHQIFGAAFKSL
FGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA
- 15 isol-ARB15076.AHF49784.1.Central_African_Republic/291-788 polyprotein [Zika virus].
isol-ARB15076.AHF49784.1.Central_African_Republic polyprotein [Zika virus].
tr|W0G4H6|W0G4H6_9FLAV Genome polyprotein [Zika virus] (SEQ ID NO: 14)
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
20 MIVNDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPWHAGADT
GTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRFSGHLKCRKMDKRLKGVSY
LCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMMLELDP
FGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGIHQIFGAAFKSL
FGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA
- 25 isol-IbH_30656.AEN75265.1.Nigeria/291-788 polyprotein, partial [Zika virus]. isol-
IbH_30656.AEN75265.1.Nigeria polyprotein, partial [Zika virus].
tr|H8XX12|H8XX12_9FLAV Genome polyprotein (Fragment) [Zika virus] (SEQ ID NO: 15)
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPWHAGADT
ETPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRFSGHLKCRKMDKRLKGVSY
LCTAAFTFTKVPAETLHGTVTVEVQYAGRDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMMLELDP
35 FGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGIHQIFGAAFKSL
FGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA
- 40 ArB1362.AHL43500.1.-/291-794 polyprotein [Zika virus]. ArB1362.AHL43500.1.-
polyprotein [Zika virus]. tr|W8Q6T3|W8Q6T3_9FLAV Genome polyprotein [Zika virus]
(SEQ ID NO: 16)
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDXXXXXXNRAEVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLP
45 HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRFSGHLKCRKMDKRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPFPGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA
- 50 ArD128000.AHL43502.1.-/291-794 polyprotein [Zika virus]. ArD128000.AHL43502.1.-
polyprotein [Zika virus]. tr|W8QIT7|W8QIT7_9FLAV Genome polyprotein [Zika virus]
(SEQ ID NO: 17)

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IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MXXXXXGHETDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDSDLYYLTMMNKHRLVRKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKLRL
5 KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGDKKITHHWLKKGSSIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGVHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

10 ArD158095.AHL43505.1.-/291-794 polyprotein, partial [Zika virus].
ArD158095.AHL43505.1.- polyprotein, partial [Zika virus]. tr|W8Q6T7|W8Q6T7_9FLAV
Genome polyprotein (Fragment) [Zika virus] (SEQ ID NO: 18)

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
15 MIVNDIGHETDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDSDLYYLTMMNKHRLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGIHQIFG
20 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

ArD158084.AHL43504.1.-/291-794 polyprotein [Zika virus]. ArD158084.AHL43504.1.-
polyprotein [Zika virus]. tr|W8Q7Q3|W8Q7Q3_9FLAV Genome polyprotein [Zika virus]
(SEQ ID NO: 19)

25 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDIGHETDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDSDLYYLTMMNKHRLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
30 LEELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

isol-ARB13565.AHF49783.1.Central_African_Republic/291-794 polyprotein [Zika virus].
35 isol-ARB13565.AHF49783.1.Central_African_Republic polyprotein [Zika virus].
tr|W0G1G4|W0G1G4_9FLAV Genome polyprotein [Zika virus] (SEQ ID NO: 20)

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDIGHETDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDSDLYYLTMMNKHRLVHKEWFHDIPLPW
40 HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGVHQIFG
30 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

45 isol-ARB7701.AHF49785.1.Central_African_Republic/291-794 polyprotein [Zika virus].
isol-ARB7701.AHF49785.1.Central_African_Republic polyprotein [Zika virus].
tr|W0G5P7|W0G5P7_9FLAV Genome polyprotein [Zika virus] (SEQ ID NO: 21)

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
50 MIVNDIGHETDENRAKVEVTPNSPRAEATLGGFSGSLGLDCEPRTGLDSDLYYLTMMNKHRLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGVFNSLGKGVHQIFG
55 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

isol-ArD_41519.AEN75266.1.Senegal/291-794 polyprotein, partial [Zika virus]. isol-ArD_41519.AEN75266.1.Senegal polyprotein, partial [Zika virus].

5 tr|H8XX13|H8XX13_9FLAV Genome polyprotein (Fragment) [Zika virus] (SEQ ID NO: 22)
 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
 TQGEAYLDKQSDTYVCKRTLVDGRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
 MIVNDTGHETDENRAKVEVTPNSPRAEATLGGFSGSLGDLCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
 HAGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKRLRL
 10 KGVSYSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
 LELDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFG
 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

15 MR766-NIID.BAP47441.1.Uganda/291-794 polyprotein [Zika virus]. MR766-NIID.BAP47441.1.Uganda polyprotein [Zika virus]. tr|A0A090BCI3|A0A090BCI3_9FLAV
 Genome polyprotein [Zika virus] (SEQ ID NO: 23)
 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
 TQGEAYLDKQSDTYVCKRTLVDGRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
 20 MTVNDIGYETDENRAKVEVTPNSPRAEATLGGFSGSLGDLCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
 HAGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKRLRL
 KGVSYSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRITANPVI TESTENSKMM
 LELDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFG
 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

25

LC002520.1/326-829 Zika virus genomic RNA, strain: MR766-NIID, Uganda, polyprotein, from complete genome (SEQ ID NO: 24)

30 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
 TQGEAYLDKQSDTYVCKRTLVDGRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
 MTVNDIGYETDENRAKVEVTPNSPRAEATLGGFSGSLGDLCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
 HAGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKRLRL
 KGVSYSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRITANPVI TESTENSKMM
 35 LELDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFG
 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

isol-MR_766.AEN75263.1.Uganda/291-794 polyprotein, partial [Zika virus].

40 MR_766.AMK02027.1.Uganda polyprotein [Zika virus]. isol-MR_766.AEN75263.1.Uganda
 polyprotein, partial [Zika virus]. MR_766.AMK02027.1.Uganda polyprotein [Zika virus].
 tr|H8XX10|H8XX10_9FLAV (SEQ ID NO: 25)

IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
 TQGEAYLDKQSDTYVCKRTLVDGRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
 MIVNDTGYETDENRAKVEVTPNSPRAEATLGGFSGSLGDLCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
 45 HAGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRKMDKRLRL
 KGVSYSLCTAAFTFTKVPATLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRITANPVI TESTENSKMM
 LELDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFG
 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

50

ArD7117.AHL43501.1.-/291-794 polyprotein [Zika virus]. ArD7117.AHL43501.1.-
 polyprotein [Zika virus]. tr|W8R1T1|W8R1T1_9FLAV Genome polyprotein [Zika virus]
 (SEQ ID NO: 26)

55 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
 TQGEAYLDKQSDTYVCKRTLVDGRGWNGCGLFGKGS�VTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG

PAT018/EP/PR

MIVNDIGHETDENRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPW
HAGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRCLKMDKLRL
KGVSYSLCTAVCTAAKVPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPFPGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVFNSLGKGIHQIFG
5 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

AY632535.2/326-825 NC_012532.1 Zika virus strain MR_766, Uganda, polyprotein, from
complete genome (SEQ ID NO: 27)

10 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIGYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGA
DTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRCLKMDKLRLKGV
YSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKIPVQMAVDMQTLTPVGRITANPVI TESTENSKMMLELD
15 PPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVFNSLGKGIHQIFGA
SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

MR_766.AAV34151.1.Uganda/291-790 polyprotein [Zika virus]

20 MR_766.AAV34151.1.Uganda polyprotein [Zika virus]. MR_766.YP_002790881.1.Uganda
flavivirus polyprotein [Zika virus]. MR_766.YP_002790881.1.Uganda flavivirus polyprotein
[Zika virus]. tr|Q32ZE1|Q32ZE1_9FL (SEQ ID NO: 28)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
25 MIGYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGA
DTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRCLKMDKLRLKGV
YSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKIPVQMAVDMQTLTPVGRITANPVI TESTENSKMMLELD
PPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVFNSLGKGIHQIFGA
30 SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

MR_766.YP_009227198.1.Uganda/1-500 envelope protein E [Zika virus] (SEQ ID NO: 29)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
35 MIGYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGA
DTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRCLKMDKLRLKGV
YSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPKIPVQMAVDMQTLTPVGRITANPVI TESTENSKMMLELD
PPFGDSYIVIGVGDKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVFNSLGKGIHQIFGA
40 SLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVMIFLSTAVSA

KU681081.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/THA/2014/SV0127- 14,
Thailand, polyprotein, from complete genome (SEQ ID NO: 30)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGSVLTCAKFTCSKKMTGKSIQPENLEYRIMLSVHGSQHSG
45 MIVNDIGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPW
HTGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRCLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TEGTENSKMM
LELDPFPGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVLNSLGKGIHQIFG
50 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

isol-Zika_virus%H.sapiens-tc%THA%2014%SV0127- 14.AMD61710.1.Thailand/291-794
polyprotein [Zika virus]. isol-Zika_virus%H.sapiens-tc%THA%2014%SV0127-
55 _14.AMD61710.1.Thailand polyprotein [Zika virus] (SEQ ID NO: 31)

PAT018/EP/PR

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HTGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRCLKMDKLRL
5 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGVLSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

- 10 CK-ISL_2014.AIC06934.1.Cook_Islands/1-504 E protein, partial [Zika virus].
tr|A0A060H177|A0A060H177_9FLAV E protein (Fragment) OS=Zika virus GN=E PE=4
SV=1 (SEQ ID NO: 32)

15 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRCLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
20 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

Natal_RGN.AMB18850.1.Brazil:_Rio_Grande_do_Norte,_Natal/291-794 polyprotein [Zika
virus]. Natal_RGN.AMB18850.1.Brazil:_Rio_Grande_do_Norte,_Natal polyprotein [Zika
virus] (SEQ ID NO: 33)

25 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRCLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
30 LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

- 35 isol-Si323.AMC37200.1.Colombia/1-504 polyprotein, partial [Zika virus]. isol-
Si322.AMC37201.1.Colombia polyprotein, partial [Zika virus]. isol-
Si322.AMC37201.1.Colombia polyprotein, partial [Zika virus]. isol-
Si323.AMC37200.1.Colombia polyprotein, partial [Zika virus] (SEQ ID NO: 34)

40 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRCLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
45 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

KU707826.1/317-820 Zika virus isolate SSABR1, Brazil, complete genome (SEQ ID NO:
35)

50 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDGRWGNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRCLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
55 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

- KU509998.1/326-829 Zika virus strain Haiti/1225/2014, Haiti, polyprotein, from complete genome (SEQ ID NO: 36)
- 5 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRKMDKRLRL
10 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA
- isol-GDZ16001.AML82110.1.China/291-794 polyprotein [Zika virus]. isol-GDZ16001.AML82110.1.China polyprotein [Zika virus] (SEQ ID NO: 37)
- 15 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRKMDKRLRL
20 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA
- 25 BeH819015.AMA12085.1.Brazil/291-794 polyprotein [Zika virus].
BeH819966.AMA12086.1.Brazil polyprotein [Zika virus]. isol-
SSABR1.AMH87239.1.Brazil polyprotein [Zika virus]. isol-SSABR1.AMH87239.1.Brazil
polyprotein [Zika virus]. BeH819966.AMA12086.1.Brazil polyprotein [Zika virus] (SEQ ID
NO: 38)
- 30 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRKMDKRLRL
35 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA
- 40 MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique/291-794 polyprotein [Zika
virus]. MRS_OPY_Martinique_PaRi_2015.AMC33116.1.Martinique polyprotein [Zika
virus] (SEQ ID NO: 39)
- 45 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRKMDKRLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA
- 50 KU501215.1/308-811 Zika virus strain PRVABC59, Puerto Rico, polyprotein, from complete
genome (SEQ ID NO: 40)
- 55 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDGRWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW

PAT018/EP/PR

HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFAEATVRGAARMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

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Haiti%1225%2014.AMB37295.1.Haiti/291-794 polyprotein [Zika virus].

Haiti%1225%2014.AMB37295.1.Haiti polyprotein [Zika virus] (SEQ ID NO: 41)

10 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
15 LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFAEATVRGAARMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

KU527068.1/308-811 Zika virus strain Natal RGN, Brazil: Rio Grande do Norte, Natal,
polyprotein, from complete genome (SEQ ID NO: 42)

20 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
25 LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFAEATVRGAARMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

isol-Z1106027.ALX35662.1.Suriname/5-508 polyprotein, partial [Zika virus]. isol-

30 Z1106027.ALX35662.1.Suriname polyprotein, partial [Zika virus] (SEQ ID NO: 43)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRL
35 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFAEATVRGAARMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

40 isol-FLR.AMM39804.1.Colombia:_Barranquilla/291-794 polyprotein [Zika virus]. isol-

FLR.AMM39804.1.Colombia:_Barranquilla polyprotein [Zika virus] (SEQ ID NO: 44)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
45 HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLSSGHLKCRKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFAEATVRGAARMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

50

PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.Canada/254-757 polyprotein, partial
[Zika virus]. PLCal_ZV_isol-From_Vero_E6_cells.AHL37808.1.Canada polyprotein, partial
[Zika virus]. tr|A0A096XFQ2|A0A096XFQ2_9FLAV Genome polyprotein (Fragment) [Zika
virus] (SEQ ID NO: 45)

PAT018/EP/PR

5 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

10 BeH818995.AMA12084.1.Brazil/291-794 polyprotein [Zika virus].
BeH818995.AMA12084.1.Brazil polyprotein [Zika virus] (SEQ ID NO: 46)
BeH815744.AMA12087.1.Brazil polyprotein [Zika virus]. BeH815744.AMA12087.1.Brazil
polyprotein [Zika virus].

15 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
20 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

H%PF%2013.AHZ13508.1.French_Polynesia/291-794 polyprotein [Zika virus].

H%PF%2013.AHZ13508.1.French_Polynesia polyprotein [Zika virus].

25 tr|A0A024B7W1|A0A024B7W1_9FLAV Genome polyprotein [Zika virus] (SEQ ID NO: 47)
IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLLSSGHLKCRLLKMDKLRL
30 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

35 PRVABC59.AMC13911.1.Puerto_Rico/291-794 polyprotein [Zika virus].
PRVABC59.AMC13911.1.Puerto_Rico polyprotein [Zika virus] (SEQ ID NO: 48)

40 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

45 KU321639.1/326-829 Zika virus strain ZikaSPH2015, Brazil, polyprotein, from complete
genome (SEQ ID NO: 49)

50 IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAAGRLLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
55 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

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ZikaSPH2015.ALU33341.1.Brazil/291-794 polyprotein [Zika virus].

ZikaSPH2015.ALU33341.1.Brazil polyprotein [Zika virus] (SEQ ID NO: 50)

5 IRCIGVSNRDFVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAQGRLLSSGHLKCRLLKMDKLRL
KGVSYSLSCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
10 LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAQMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

103344.AMC13912.1.Guatemala/291-794 polyprotein [Zika virus].

15 103344.AMC13912.1.Guatemala polyprotein [Zika virus]. 8375.AMC13913.1.Guatemala
polyprotein [Zika virus]. 8375. AMC13913.1.Guatemala polyprotein [Zika virus]. (SEQ ID
NO: 51)

20 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEIRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAQGRLLSSGHLKCRLLKMDKLRL
KGVSYSLSCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAQMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

25 isol-Brazil-ZKV2015.AMD16557.1.Brazil/291-794 polyprotein [Zika virus]. isol-Brazil-
ZKV2015.AMD16557.1.Brazil polyprotein [Zika virus] (SEQ ID NO: 52)

30 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAQGRLLSSGHLKCRLLKMDKLRL
KGVSYSLSCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAQMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
35 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

KU497555.1/308-811 Zika virus isolate Brazil-ZKV2015, Brazil, polyprotein, from complete
genome (SEQ ID NO: 53)

40 IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGTQEGAVHTALAGALEAEMDGAQGRLLSSGHLKCRLLKMDKLRL
KGVSYSLSCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM
45 LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAQMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

isol-ZJ03.AMM39806.1.China/291-794 polyprotein [Zika virus]. isol-

50 ZJ03.AMM39806.1.China polyprotein [Zika virus] (SEQ ID NO: 54)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVDRGWNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNKHVLVHKEWFHDIPLPW
HAGADTGTPHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAQGRLLSSGHLKCRLLKMDKLRL
KGVSYSLSCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRLLITANPVI TESTENSKMM

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LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGARRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

5 isol-FSS13025.AFD30972.1.Cambodia/291-794 polyprotein, partial [Zika virus]. isol-
FSS13025.AFD30972.1.Cambodia polyprotein, partial [Zika virus].
tr|H9A910|H9A910_9FLAV Genome polyprotein (Fragment) [Zika virus] (SEQ ID NO: 55)
IRCI GVS NRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
10 MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

15

isol-Z1106032.ALX35660.1.Suriname/291-794 polyprotein, partial [Zika virus]. isol-
Z1106032.ALX35660.1.Suriname polyprotein, partial [Zika virus] (SEQ ID NO: 56)
IRCI GVS NRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCP
20 TQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
25 AAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGGVLIFLSTAVSA

isol-Z1106033.ALX35659.1.Suriname/291-794 polyprotein [Zika virus]. isol-
Z1106033.ALX35659.1.Suriname polyprotein [Zika virus] (SEQ ID NO: 57)
IRCI GVS NRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCP
30 TQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKRLKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVI TESTENSKMM
35 LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGGVLIFLSTAVSA

isol-BeH828305.AMK49165.1.Brazil/291-794 polyprotein [Zika virus]. isol-
BeH828305.AMK49165.1.Brazil polyprotein [Zika virus] (SEQ ID NO: 58)
IRCI GVS NRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCP
40 TQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPLPW
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKRLKMDKLRL
45 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDTQTLPVGRLITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

isol-GD01.AMK79468.1.China/291-794 polyprotein [Zika virus]. isol-
GD01.AMK79468.1.China polyprotein [Zika virus] (SEQ ID NO: 59)
IRCI GVS NRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD RGWNGCGLFGKGS LVTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNGTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKHLVHKEWFHDIPLPW
50 HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKRLKMDKLRL

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KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRS GSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

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isol-Z1106031.ALX35661.1.Suriname/291-794 polyprotein, partial [Zika virus]. isol-
Z1106031.ALX35661.1.Suriname polyprotein, partial [Zika virus] (SEQ ID NO: 60)

10 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKH W L V H K E W F H D I P L P W
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCR LKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVLAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRS GSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFG
15 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

ACD75819.1.Micronesia/291-794 polyprotein [Zika virus]. ACD75819.1.Micronesia
polyprotein [Zika virus]. tr|B3U3M3|B3U3M3_9FLAV Genome polyprotein [Zika virus]
(SEQ ID NO: 61)

20 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKH W L V H K E W F H D I P L P W
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCR LKMDKLRL
25 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRS GSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

KU681082.3/308-811 Zika virus isolate Zika virus/H.sapiens-tc/PHL/2012/CPC-0740,
Philippines, polyprotein, from complete genome (SEQ ID NO: 62)

30 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKH W L V H K E W F H D I P L P W
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCR LKMDKLRL
35 KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRS GSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

40 isol-Zika_virus%H.sapiens-tc%PHL%2012%CPC-0740.AMD61711.1.Philippines/291-794
polyprotein [Zika virus]. isol-Zika_virus%H.sapiens-tc%PHL%2012%CPC-
0740.AMD61711.1.Philippines polyprotein [Zika virus] (SEQ ID NO: 63)

45 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTT VSNMAEVR SYCYEASISDMASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G
MIVNDTG HETDENRAKVEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MN NKH W L V H K E W F H D I P L P W
HAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCR LKMDKLRL
KGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWHRS GSTIGKA FEATVRGAKRMAVLGDTAWDFG SVGGALNSLGKGIHQIFG
50 AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

isol-BeH823339.AMK49164.2.Brazil/291-794 polyprotein [Zika virus]. isol-
BeH823339.AMK49164.2.Brazil polyprotein [Zika virus] (SEQ ID NO: 64)

55 IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVSTTVSNMAEVR SYCYEATISDIASDSRCP
TQGEAYLDKQSDTQYVCKRTLVD R G W G N G C G L F G K G S L V T C A K F A C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G

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MIVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNNKHVLVHKEWFHDIPLPW
HAGADTGTTPHWNKEALVEFKDAHAKRQTAVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAEATLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
5 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSA

isol-P6-740.AEN75264.1.Malaysia/291-794 polyprotein, partial [Zika virus]. isol-P6-
740.AEN75264.1.Malaysia polyprotein, partial [Zika virus]. tr|H8XX11|H8XX11_9FLAV
10 Genome polyprotein (Fragment) [Zika virus] (SEQ ID NO: 65)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MIVNDXGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNNKHVLVHKEWFHDIPLPW
HAGADTGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLLKMDKLRL
15 KGVSYSLCTAAFTFTKIPAEATLHGTVTVEVQYAGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGDKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIFG
AAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLALGGVLIFLSTAVSA

20 KU744693.1/326-829 Zika virus isolate VE_Ganxian, China, polyprotein, from complete
genome (SEQ ID NO: 66)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
MLVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNNKHVLAHKEWFHDIPLPW
25 HAGAATGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAEATVDGTVTVEGQYGGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIIG
AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSG

30 isol-VE_Ganxian.AMK79469.1.China/291-794 polyprotein [Zika virus]. isol-
VE_Ganxian.AMK79469.1.China polyprotein [Zika virus] (SEQ ID NO: 67)

IRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTMAQDKPTVDIELVTTTTSNMAEVRSYCYEASISDMASDSRCP
TQGEAYLDKQSDTYVCKRTLVDRGWNGCGLFGKGSVLTCAKFACSKKMTGKSIQPENLEYRIMLSVHGSQHSG
35 MLVNDTGHETDENRAKVEITPNSPRAEATLGGFSGSLGLDCEPRTGLDFSDLYYLTMMNNKHVLAHKEWFHDIPLPW
HAGAATGTTPHWNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLLKMDKLRL
KGVSYSLCTAAFTFTKIPAEATVDGTVTVEGQYGGTDGPKVPAQMAVDMQTLTPVGRITANPVI TESTENSKMM
LELDPPFGDSYIVIGVGEKKITHHWRSGSTIGKAFAEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIIG
40 AAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLALGGVLIFLSTAVSG

In some embodiments, the Zika virus has an envelope (E) protein with an amino acid
sequence provided by any one of SEQ ID NO: 12-67. In some embodiments, the E protein
sequence is at least 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9%
identical to the E protein sequence provided by any one of the amino acid sequences SEQ ID
45 NO: 12-67.

The terms "identical" or percent "identity" in the context of two or more nucleic acids
or amino acid sequences refer to two or more sequences or subsequences that are the same.
Two sequences are "substantially identical" if two sequences have a specified percentage of
amino acid residues or nucleotides that are the same (*e.g.*, at least 80%, 85%, 90%, 95%,

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96%, 97%, 98%, or 99% identity) over a specified region or over the entire sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length. In some embodiments, the identity exists over the length of a protein, such as the E protein.

For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. Methods of alignment of sequences for comparison are well known in the art. See, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443, 1970, by the search for similarity method of Pearson and Lipman. Proc. Natl. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, Jalview and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group. 575 Science Or., Madison. WI), by multi sequence alignment implementation using *e.g.* CLUSTALW (Larkin et al., (2007). Bioinformatics, 23, 2947-2948.) or MAFFT (Katoh & Toh 2008 Briefings in Bioinformatics 9:286-298), or by manual alignment and visual inspection (see. *e.g.*, Brent et al., Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (Ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977; and Altschul et al., J. Mol. Biol. 215:403-410, 1990, respectively.

Aspects of the disclosure relate to methods of producing a Zika virus in Vero tissue culture cells. Vero cells are a commonly used tissue culture cell line derived from the kidney of an African green monkey. The Vero cells used in the methods described herein are the VERO (WHO) cell line, obtained from the Health Protection Agency general cell collection under catalogue number 88020401.

Vero cells can be grown to confluent monolayers, for example in tissue culture flasks; in suspension (on microcarriers), for example in roller bottles; or in any other cell culture system for viral production. In some embodiments, the Vero cells are grown in a bioreactor for viral production. For plaque assays or plaque reduction neutralization test (PRNT), Vero

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cells are grown in monolayers in tissue culture flasks, dishes, or wells of a plate. To infect the Vero cells with the Zika virus, the culture medium is inoculated with Zika virus and the cells are incubated with the Zika virus for a period of time. The cells may be washed after inoculation to remove any virus that did not adsorb to the cells in a given amount of time.

5 The methods provided herein involve passaging the Zika virus in Vero cells. As used herein, the terms “passage” or “passaging” refer to infecting a population of Vero cells with Zika virus and subsequently inoculating a second population of Vero cells with Zika virus produced by infection of the first Vero cell population. In some embodiments, a portion of the culture medium from the infected Vero cells (containing Zika virus that was released
10 from the infected cells) is used to inoculate a second population of Vero cells. This is referred to as one passage or one round of passaging. The passaging may be performed serially, for example, a portion of the culture medium from the infected second population of Vero cells is used to inoculate a third population of Vero cells, and so on. In some embodiments, Zika virus obtained from a single plaque is used to inoculate another
15 population of cells.

 In some embodiments, the Zika virus is passaged in Vero cells several times, such as at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times. In some embodiments, the Zika virus is passaged in Vero cells at least 4 times. In some embodiments, the Zika virus is passaged in
20 Vero cells at least 30 times.

 In some embodiments, the Vero cells are incubated for at least 2 days after inoculation with the Zika virus to allow for viral production, prior to passaging. In some embodiments, the Vero cells are incubated for at least 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 days e.g. at least 20 days after inoculation with the Zika virus prior to passaging. The number
25 of days the Vero cells are incubated after viral inoculation may depend on factors such as the multiplicity of infection used to inoculate the cells and the viral titer desired in the culture medium. Serial passaging of the Zika virus in Vero cells may result in generation of a Vero cell adapted Zika virus strain.

 The culture medium from the infected Vero cells may be harvested (collected) to
30 obtain the Zika virus. In some embodiments, the culture medium is harvested from infected Vero cells and is replaced with fresh culture medium, which is then harvested after another period of time. In some embodiments, the culture medium harvested from infected Vero cells

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is pooled from independent Vero cell cultures and/or from independent days. Harvesting can be repeated up to 4 times by 8 or 9 days post infection, for example, and result in a high yield of virus per unit cell culture. The harvested culture medium can be stored at +4°C prior to purification of the virus from the culture medium.

5 In some embodiments, debris from infected and lysed Vero cells may be removed from the harvested culture medium, referred to as a “clarification” of the culture medium. The harvested culture medium may be clarified by common methods known in the art, such as low-speed centrifugation, for example, at 1500 g for 10 min, and/or by filtration through a filter of pore size of 0.45 µm. The harvested culture medium can be stored at +4°C prior to
10 concentration.

 To concentrate the titer of the virus in the harvested culture medium, it may be subjected to concentration by any method known in the art. For example, the harvested culture medium may be concentrated by methods including, without limitation, ultrafiltration, ultracentrifugation, centrifugal concentrator, vacuum centrifugation, and lyophilization. In
15 some embodiments, the harvested culture medium is concentrated by ultrafiltration and the retentate containing the Zika virus is collected. In some embodiments, the harvested culture medium is concentrated by precipitation in which polyethylene glycol (PEG) 8000 is dissolved in the culture medium (up to 10%) and the precipitate is dissolved in a buffer, for example phosphate-buffered saline (PBS, pH 7.0).

20 The harvested culture medium may be precipitated to produce a Zika virus supernatant. In some embodiments, the harvested culture medium is precipitated to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. In some embodiments, the harvested culture medium is concentrated prior to precipitation. In some embodiments, the harvested culture medium is precipitated by
25 adding protamine sulfate to the harvested culture medium and incubating the mixture, for example at +4°C or on ice. In some embodiments, the harvested culture medium is treated with benzonase to remove Vero cell DNA and other undesired material, such as Vero cell debris, from the harvested culture medium. In some embodiments, the precipitated culture medium is centrifuged to collect precipitated material and the supernatant containing the Zika
30 virus, referred to as a “Zika virus supernatant,” is collected.

 The Zika virus supernatant may be further purified after precipitation, for example density gradient ultracentrifugation. In some embodiments, the Zika virus supernatant is

further purified by sucrose gradient. Fractions may be collected from the sucrose gradients and assayed for presence of the Zika virus. Methods for assaying for virus positive fractions include plaque assay, hemagglutination assay, polyacrylamide gel electrophoresis, and antigen assays such as Western blotting and ELISA's. The fractions containing Zika virus
5 may be pooled based on titer of the virus and level of other impurities. The level or amount of impurities present in the Zika virus supernatant can be estimated by testing for Vero cell DNA and/or Vero cell protein.

To achieve a safe vaccine or composition for the administration to subjects, the Zika virus supernatant is inactivated. As used herein, the terms "inactivated" and "optimally
10 inactivated" may be used interchangeably and refer to a process (or its result) by which the virus is rendered unable to infect a host cell (non-infectious), but that does not affect or substantially affect the antigenicity of the virus, for example, the immunogenic antigens exposed on the surface of the Zika virus are able to stimulate an immune response in a subject (*e.g.*, antigen-specific antibodies). By "does not affect or substantially affect the
15 antigenicity of the virus" is meant that the inactivated Zika virus retains at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even essentially 100% of the antigenicity of a Zika virus that is not subjected to inactivation.

A variety of methods are known in the art for inactivating viruses. In some
20 embodiments, the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

In some embodiments, the inactivating is by chemical inactivation and involves contacting the Zika virus with one or more chemical inactivation agent for a period of time under conditions such that the virus is inactivated but the antigenic epitopes are substantially
25 intact. In some embodiments, the Zika virus is inactivated for a period of time that is longer than is required to completely inactivate the Zika virus. In some embodiments, the Zika virus supernatant is inactivated for the number of days required to inactivate the virus plus at least one additional day. Samples of the Zika virus supernatant may be taken at one or more times throughout the inactivation process and assessed for viral viability (infectivity) by any
30 method known in the art, such as by infecting a monolayer of host cells (*i.e.*, plaque assay). Using such a procedure, the period of time that is required to completely inactivate the Zika

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virus can be determined, and a longer period of time is selected to ensure complete inactivation.

In some embodiments, the Zika virus is contacted with a chemical inactivation agent for between 1 day and 50 days, between 2 days and 40 days, between 2 days and 30 days, between 2 days and 20 days, between 2 days and 10 days, between 3 days and 9 days, between 4 days and 8 days, between 5 days and 7 days, between 2 days and 5 days, or between 5 and 10 days. In some embodiments, the Zika virus is contacted with one or more chemical inactivation agents for at least 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 32 days, 33 days, 34 days, 35 days, 36 days, 37 days, 38 days, 39 days, 40 days, 41 days, 42 days, 43 days, 44 days, 45 days, 46 days, 47 days, 48 days, 49 days, or at least 50 days.

In some embodiments, the chemical inactivation is performed at about +5°C, +10°C, +15°C, +20°C, +25°C, +30°C, +35°C, +40°C, or about +45°C. In some embodiments, the chemical inactivation is performed at about +4°C. In some embodiments, the chemical inactivation is performed at about +22°C.

Any chemical inactivation agent known in the art may be suitable for inactivating the Zika virus in the methods described herein. It will be appreciated by one of skill in the art that factors such as the chemical inactivation agent and the temperature at which inactivation is performed may affect the length of time (number of days) required to completely inactivate the Zika virus. Examples of chemical inactivation agents include, without limitation, formaldehyde, enzymes, β -propiolactone, ethanol, trifluoroacetic acid, acetonitrile, bleach, urea, guanidine hydrochloride, tri-n-butyl phosphate, ethylene-imine or a derivatives thereof, and organic solvents such as Tween, Triton, sodium deoxycholate, and sulphohetain.

In some embodiments, the inactivating agent is neutralized after chemical inactivation of the virus. In some embodiments, the inactivating agent is formaldehyde and is neutralized after chemical inactivation using sodium thiosulphate or sodium metabisulfite.

In some embodiments, the Zika virus is inactivated by thermal inactivation. In some embodiments, the thermal inactivation involves exposing the Zika virus to heat, such as dry heat or vapor heat, for a period of time. In some embodiments, the thermal inactivation involves exposing the Zika virus to temperatures of about +40°C, +45°C, +50°C, +55°C,

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+60°C, +65°C, +70°C, +75°C, +80°C, +85°C, +90°C, +95°C, or about +100°C. In some embodiments, the Zika virus is exposed to heat for at least 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, about 96 hours, or longer. A preferred thermal inactivation involves exposing the Zika virus to temperatures of about +56°C for 60 minutes.

In some embodiments, the Zika virus is inactivated by exposing the virus to acidic or alkaline conditions for a period of time such that the virus is completely inactivated. The pH of a Zika virus preparation may be adjusted to a desired pH, for example by the addition of an acid, a base, or a buffer with a particular pH to the virus preparation. In some embodiments, the virus is inactivated at an acidic pH of about 2, 2.5, 3, 3.5, 4, 4.5, 5 or about 5.5. In other embodiments, the virus is inactivated at an alkaline pH of about 8, 8.5, 9, 9.5, 10, or about 10.5.

In some embodiments, the Zika virus is inactivated using UV inactivation. UV inactivation involves exposing the Zika virus to energy-rich radiation, such as UV-A, UV-B, or UV-C light for a period of time.

It will be appreciated that any two or more methods of inactivation may be combined and performed concurrently or serially.

The inactivated Zika virus may be subsequently dialyzed to remove any undesired material, including the inactivating agent and any neutralizing agent, and/or to replace the buffer with a buffer that is pharmaceutically acceptable for administration to subjects. In some embodiments, the inactivated Zika virus is dialyzed with PBS. In addition or alternatively, the inactivated Zika virus may be filtered, such as sterile filtered, through a 0.22 µm filter.

Any of the methods or uses described herein may be for the prevention of a Zika virus infection in a subject. As used herein, the terms “prevent,” and “preventing,” include the administration of a Zika virus vaccine or composition to a subject to reduce, or delay the onset of the manifestation of clinical or subclinical symptoms, complications, pathologies or biochemical indicia of a disease or infection, or to reduce or inhibit the spread/transmission of the Zika virus. As used herein, antigen(s), such as an inactivated Zika virus, that is administered to a subject prophylactically (e.g., prior to infection) may be referred to as a vaccine.

As described herein Zika virus may cause any of a variety of symptoms upon infection of a subject, and is generally characterized by mild fever; rash (exanthema) on face, neck trunk, upper arms; headache; sensitivity to light; non-inflammatory joint pain; conjunctivitis; lack of appetite; diarrhea; abdominal pain; and/or dizziness. Zika virus infection during pregnancy is likely associated with microcephaly in the fetus/neonate. There is also a probable association between the onset of Guillain-Barré syndrome or symptoms thereof. Diagnosis of Zika virus infection in subjects exposed to Zika virus or suspected of being exposed to Zika virus involves detecting the presence of virus-specific antibodies and/or molecular testing, such as PCR or real-time PCR detection of Zika virus.

Provided herein are methods for administering a dose of a therapeutically effective amount of a Zika virus vaccine to a subject in need thereof. In some embodiments, the subject is a mammalian subject, such as a human, non-human primate, rodent, rabbit, sheep, dog, cat, horse, or cow. In some embodiments, the subject is a mouse. In some embodiments, the subject is a human subject, such as a child, an adult, or an elderly adult. In some embodiments, the subject is a female subject. In some embodiments, the subject is pregnant or planning on becoming pregnant. In some embodiments, the subject is at risk of being exposed to Zika virus. In some embodiments, the subject is living in or traveling to an area where Zika virus is present or is thought to be present. In some embodiments, the subject is living in or traveling to an area that is experiencing a Zika virus infection outbreak. In some embodiments, the subject is living in or traveling to an area where an arthropod vector capable of transmitting the Zika virus vector is present or is thought to be present.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject in a therapeutically effective amount or a dose of a therapeutically effective amount. As used herein, a “therapeutically effective amount” of vaccine is any amount that results in a desired response or outcome in a subject, such as those described herein, including but not limited to prevention of infection, an immune response or an enhanced immune response to Zika virus, or prevention or reduction of symptoms associated with Zika disease.

In some embodiments, the therapeutically effective amount of a Zika virus vaccine or composition described herein is an amount sufficient to generate antigen-specific antibodies (*e.g.*, anti-Zika virus antibodies). In some embodiments, the therapeutically effective amount is sufficient to seroconvert a subject with at least 70% probability. In some embodiments, the

therapeutically effective amount is sufficient to seroconvert a subject with at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least 99% probability. Whether a subject has been seroconverted can be assessed by any method known in the art, such as obtaining a serum sample from the subject and performing an assay to detect anti-Zika virus antibodies. In some embodiments, a subject is seroconverted if a serum sample from the subject contains an amount of anti-Zika virus antibodies that surpasses a threshold or predetermined baseline. A subject is generally considered seroconverted if there is at least a 4-fold increase in anti-Zika virus antibodies (*i.e.*, anti-Zika E protein IgG antibodies) present in a serum sample from the subject as compared to a serum sample previously taken from the same subject.

In some embodiments, seroconversion of a subject is assessed by performing a plaque reduction neutralization test (PRNT). Briefly, PRNT is used to determine the serum titer required to reduce the number of Zika virus plaques by 50% (PRNT50) as compared to a control serum/antibody. The PRNT50 may be carried out using monolayers of Vero cells or any other cell type/line that can be infected with Zika virus. Sera from subjects are diluted and incubated with live, non-inactivated Zika virus. The serum/virus mixture may be applied to the Vero cells and incubated for a period of time. Plaques formed on the Vero cell monolayers are counted and compared to the number of plaques formed by the Zika virus in the absence of serum or a control antibody. A threshold of neutralizing antibodies of 1:10 dilution of serum in a PRNT50 is generally accepted as evidence of protection (Hombach *et al.* Vaccine (2005) 23:5205-5211).

In some embodiments, the Zika virus may be formulated for administration in a composition, such as a pharmaceutical composition. The term “pharmaceutical composition” as used herein means a product that results from the mixing or combining of at least one active ingredient, such as an inactivated Zika virus, and one or more inactive ingredients, which may include one or more pharmaceutically acceptable excipient.

Pharmaceutical compositions of the invention, including vaccines, can be prepared in accordance with methods well known and routinely practiced in the art (see *e.g.*, Remington: The Science and Practice of Pharmacy, Mack Publishing Co. 20th ed. 2000; and Ingredients of Vaccines – Fact Sheet from the Centers for Disease Control and Prevention, *e.g.*, adjuvants and enhancers such as alum to help the vaccine improve its work, preservatives and stabilizers to help the vaccine remain unchanged (*e.g.*, albumin, phenols, glycine)). Pharmaceutical compositions are preferably manufactured under GMP conditions. Typically

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a therapeutically effective dose of the inactivated Zika virus preparation is employed in the pharmaceutical composition of the invention. The inactivated Zika virus is formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art. Dosage regimens are adjusted to provide the optimum desired response (*e.g.*, the prophylactic response).

Dosages of the active ingredients in the pharmaceutical compositions of the present invention can be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired pharmaceutical response for a particular subject, composition, and mode of administration, without being toxic to the subject. The selected dosage level depends upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the subject being treated, and like factors.

A physician, veterinarian or other trained practitioner, can start doses of the inactivated Zika virus vaccine employed in the pharmaceutical composition at levels lower than that required to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect (*e.g.*, production of anti-Zika virus antibodies) is achieved. In general, effective doses of the compositions of the present invention, for the prophylactic treatment of groups of people as described herein vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and the titer of anti-Zika virus antibodies desired. Dosages need to be titrated to optimize safety and efficacy. In some embodiments, the dosing regimen entails subcutaneous or intramuscular administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 7. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 14. In some embodiments, the dosing regimen entails subcutaneous administration of a dose of inactivated Zika virus twice, once at day 0 and once at about day 28. In some embodiments, the inactivated Zika virus is administered to the subject once.

Any of the Zika virus vaccines or compositions described herein may be administered to a subject with, prior to, or after administration of one or more adjuvants. An adjuvant is a molecule that enhances a response in a subject, such as an immune response, to an antigen or other molecule. In some embodiments, an adjuvant may stabilize an antigen or other
 5 molecule. Determining whether a Zika virus vaccine or compositions thereof are administered with an adjuvant depends on various factors (e.g., type and extent of response desired) and will be evident to one of skill in the art. In some embodiments, administering any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may enhance the production of virus neutralizing (anti-Zika
 10 virus) antibodies. In some embodiments, a subject that is administered any of the Zika virus vaccines or compositions described herein with, prior to, or after administration of an adjuvant may only require a single administration of the Zika virus vaccine or composition to be seroconverted (produce a level of anti-Zika virus antibodies). Examples of adjuvants may include, without limitation, aluminium salt (aluminium hydroxide or aluminium phosphate),
 15 calcium phosphate hydroxide, paraffin oil, killed bacteria, bacterial toxins, toxoids, subunits of bacteria, squalene, thimerosal, detergents, IL-1, IL-2, IL-12, 2-component adjuvants, such as 2-component adjuvants containing an antibacterial peptide and a TLR9 agonist (*e.g.*, IC31®), and combinations such as Freund's complete adjuvant and Freund's incomplete adjuvant. In some embodiments, the Zika virus vaccines or compositions is administered
 20 with aluminium hydroxide. In some embodiments, the inactivated Zika virus vaccine or composition is administered with aluminium phosphate salt.

In some embodiments, the adjuvant is comprised of two components. In some embodiments, the 2-component adjuvant comprises an antibacterial peptide and a TLR9 agonist. In some embodiments, the antibacterial peptide is provided by the amino acid
 25 sequence KLKL₅KLK (SEQ ID NO: 68). In some embodiments, the TLR9 agonist is a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN). In some embodiments, the I-ODN comprises the nucleic acid sequence (dIdC)₁₃ (SEQ ID NO: 69). In some embodiments, the adjuvant is IC31®. In some embodiments, the adjuvant is in nanoparticle form (See, *e.g.*, US Patent No. 8,765,148 B2, incorporated by reference in its
 30 entirety). In some embodiments, the adjuvant is IC31®, *i.e.* KLKL₅KLK (SEQ ID NO: 68) and the nucleic acid sequence (dIdC)₁₃ (SEQ ID NO: 69), in combination with an aluminium salt such as aluminium hydroxide.

The Zika virus vaccines or compositions described herein may be administered to a subject concomitantly with one or more vaccine to another infectious agent, such as another infectious agent is that present or thought to be present in the same geographic area as Zika virus. In some embodiments, the other infectious agent is one that the subject is also at risk of being in contact with. In some embodiments, the other infectious agent is transmitted by the same arthropod vector as Zika virus. In some embodiments, the other infectious agent is Japanese Encephalitis virus, Yellow Fever virus, Dengue virus and/or Chikungunya virus.

Also within the scope of the present disclosure are kits for use in prophylactically administering to a subject, for example to prevent or reduce the severity of Zika virus infection. Such kits can include one or more containers comprising a composition containing inactivated Zika virus, such as an inactivated Zika virus vaccine. In some embodiments, the kit may further include one or more additional containers comprising a second composition, such as a second vaccine. In some embodiments, the second vaccine is a vaccine for another arbovirus. In some embodiments, the second vaccine is a Dengue virus vaccine and/or a Chikungunya virus vaccine.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the composition containing inactivated Zika virus to prevent, delay the onset, or reduce the severity of Zika virus infection. The kit may further comprise a description of selecting a subject suitable for administration based on identifying whether that subject is at risk for exposure to Zika virus or contracting a Zika virus infection. In still other embodiments, the instructions comprise a description of administering a composition containing inactivated Zika virus to a subject at risk of exposure to Zika virus or contracting Zika virus infection.

The instructions relating to the use of the composition containing inactivated Zika virus generally include information as to the dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine readable instructions are also acceptable.

The kits of the present disclosure are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also

contemplated are packages for use in combination with a specific device, such as a syringe or an infusion device. The container may have a sterile access port, for example the container may be a vial having a stopper pierceable by a hypodermic injection needle. At least one active agent in the composition is an inactivated Zika virus, as described herein.

5

This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of “including,”
10 “comprising,” or “having,” “containing,” “involving,” and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those
15 of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art. Generally, nomenclatures used in connection with, and techniques of biochemistry, enzymology, molecular and cellular biology, microbiology, virology, cell or
20 tissue culture, genetics and protein and nucleic chemistry described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated.

25 The present invention is further illustrated by the following examples, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove. However, the citation
30 of any reference is not intended to be an admission that the reference is prior art.

EXAMPLES

Example 1: Adaptation of Zika Virus in Vero cells

Vero cell monolayers are inoculated with Zika virus. The infected cell cultures are
5 grown in 25 cm² culture flasks containing 5 mL of nutrient media consisting of Eagle's
minimal essential media (EMEM) supplemented with 10% fetal bovine serum (FBS) in an
atmosphere of about 5% CO₂ in air and at a temperature no higher than about +35°C. Viral
replication is monitored by microscopic observation of cytopathic effect (CPE) and various
assays for the presence of viral antigen including hemadsorption assay (HA), plaque assay,
10 and enzyme linked immunoadsorbant assay (ELISA). Zika virus is harvested at day *e.g.* day
1 and/or 2 or other day, *e.g.* 3, 4, 5, 6, 7, 8, 9, 10 post infection when the culture showed peak
of virus titer, and culture media is clarified by centrifugation to remove cell debris. The
clarified supernatant is used to inoculate additional Vero cell monolayers in order to amplify
the virus. Subsequent serial passages are conducted up to 30 passages by successive virus
15 infection, titration, and plaque-purification as described above. The viral yield in the culture
media is quantified by plaque assay.

Example 2: Virus growth and purification

For viral production and subsequent purification, Vero cells are grown in Eagle's
20 minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS). Roller bottle
cultures of Vero cell monolayers are infected with Zika virus at a multiplicity of infection
(moi) of 0.01 to 1 plaque forming units (pfu) per cell. After 2 hours of virus adsorption, the
cultures are washed 3 times with PBS and fed with EMEM without FBS and incubated at
+35°C with 5% CO₂. Infected Vero cell cultures are incubated until the virus titer reaches a
25 desired level.

The culture medium is harvested and may be pooled from independent harvest days
and then centrifuged at 8,000 rpm for 15 minutes in standard centrifuge. The supernatants
are then filtered through a 0.45 µm filter. Virus culture supernatants are concentrated by
ultrafiltration (Ultrasette, Filtron, 100k) or precipitation with PEG. The virus precipitated by
30 PEG is collected by centrifugation and resuspended in PBS or STE (10 mM Tris pH 7.2,
1 mM EDTA, 150 mM NaCl) buffer. Retentate after ultrafiltration is concentrated to 250 mL
and the cassette is washed with 100 mL of PBS. Virus concentrates are precipitated by

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chilling in the ice for 2 hours after adding 0.5-2 mg/mL of protamine sulfate. The precipitated concentrates are centrifuged at 10,000 rpm for 5 minutes in standard centrifuge to obtain the supernatant containing the virus. The concentrated viruses are purified by ultracentrifugation on sucrose gradients at 38,000 g for 18 hours.

5 Fractions are subjected to analysis by SDS-PAGE. Envelope antigens (E) may be detected by Western blotting with anti-Zika virus monoclonal antibody. Virus positive fractions are pooled and assayed for protein concentration, for example by Lowry method. Purified virus is diluted with two volumes of PBS and then filtered through a 0.22 µm filter.

The virus preparations are compared for relative purity using specific activity
10 measurements (*i.e.*, pfu/mg protein). The purity of the Zika virus preparations are estimated by assessing the preparations for any Vero cell DNA or protein.

Example 3: Virus Inactivation

Purified Zika virus preparations are inactivated with formaldehyde for preparation of
15 inactivated vaccine. Inactivation with 0.018% formaldehyde may be carried out at +22°C or +4°C for at least 2 days, samples from the viral inactivation may be taken at different time intervals to assess for viral viability by plaque assay. The antigenicity of the virus can also be monitored during inactivation by testing samples with antigen dot blot assay and probing with a polyclonal antiserum.

20 After complete viral inactivation, the formaldehyde is neutralized by the addition of 0.038% of sodium metabisulfite. Dialysis is performed using PBS and the preparations are then filtered through a 0.22 µm filter.

Example 4: Immunogenicity of Vero adapted Zika virus

25 The immunogenicity of inactivated Zika virus preparations is assessed using a mouse model of Zika infection. Groups of adult mice are immunized subcutaneously (*s.c.*) with 500, 50, or 5 ng of inactivated Zika virus with adjuvant (*e.g.* aluminium hydroxide with or without IC31®), or without adjuvant. An additional group of mice receive PBS as a negative control. Each group is administered the indicated inoculum at *t*=0 and in some cases also at three to
30 four weeks later (*t*=3/4). Beginning approximately three weeks after administration of the last immunization, serum samples are obtained from each of the mice at regular intervals. The serum samples are tested for the presence of neutralizing antibodies using PRNT.

The *in vivo* protective efficacy of the inactivated Zika virus preparations is also assessed using a mouse model of Zika infection, *i.e.* IFN-alpha/beta receptor knock-out mice (A129) (see *e.g.* Dowall et al., 4. March 2016, <http://dx.doi.org/10.1101/042358>) or blocking of the IFN-alpha/beta receptor by administration of anti-IFN-alpha/beta receptor monoclonal antibodies to C57BL/6 or BALB/c mice (see *e.g.* Pinto et al., 7. December 2011, DOI: 10.1371/journal.ppat.1002407). For protection assays, groups of 10 three- to eight-weeks-old A129, C57BL/6 or BALB/c mice are inoculated subcutaneously in the hindquarters with inactivated Zika virus with adjuvant (aluminium hydroxide) or without adjuvant at t=0. Age-matched controls are inoculated with PBS or non-specific antigens in alum. Mice are optionally boosted with a second administration of the indicated inoculation three to four weeks later. The mice are then challenged subcutaneously at three to eight weeks post immunization by inoculation with a deadly dose of live Zika virus. One day prior to challenge of C57BL/6 and BALB/c mice, they are passively administered (intraperitoneally) with anti-IFN-alpha/beta receptor monoclonal antibodies. Challenged mice are monitored daily for morbidity and mortality for up to twenty-one days. Another alternative is to challenge intracranially adult vaccinated/non-vaccinated adult mice and observe protection.

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Further more detailed aspects of the invention:

5 A1. A Zika virus vaccine comprising an optimally inactivated Zika virus particle, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability.

A2. The Zika virus vaccine of claim A1, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability.

10

A3. The vaccine of claim A1 or A2, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 1-11, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 1-11 and able to pack a virulent Zika virus.

15

A4. The vaccine of any one of claims A1-A3, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 12-67, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 12-67 and able to pack a virulent Zika virus.

20

A5. The vaccine of any one of claims A1-A4, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

25 A6. The vaccine of claim A5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent to completely inactivate the Zika virus as measured by plaque assay.

30

A7. The vaccine of claim A6, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.

A8. The vaccine of claim A7, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

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A9. The vaccine of any one of claims A5-A8, wherein the chemical activation is performed at about +4°C or about +22°C.

5 A10. The vaccine of any one of claims A1-A9, further comprising an adjuvant.

A11. The vaccine of claim A10, wherein the adjuvant is an aluminum salt adjuvant.

10 A12. The vaccine of claim A11, wherein the aluminum salt adjuvant is aluminium hydroxide or aluminium phosphate salt.

A13. The vaccine of any one of A10-A12, wherein the vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).
15

A14. The vaccine of claim A13, wherein the peptide comprises the sequence KLKL5KLK (SEQ ID NO: 68) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 69).

20 A15. The vaccine of any one of claims A1-A14, further comprising one or more pharmaceutically acceptable excipient.

B1. A kit comprising a Zika virus vaccine of any one of claims A1-A15.

25 B2. The kit of claim B1, further comprising a second vaccine.

B3. The kit of claim B2, wherein the second vaccine is a West Nile virus vaccine, a Japanese Encephalitis virus vaccine, a Yellow Fever virus vaccine, a Dengue virus vaccine or a Chikungunya virus vaccine.
30

C1. A method, comprising

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administering a first dose of a therapeutically effective amount of the Zika virus vaccine of any one of claims A1-A15 to a subject in need thereof.

5 C2. The method of claim C1, further comprising administering a second dose of a therapeutically effective amount of the Zika virus vaccine.

C3. The method of claim C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

10 C4. The method of claim C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

C5. The method of claim C1 or C2, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

15

C6. The method of any one of claims C1-C5, wherein the administering results in production of Zika virus neutralizing antibodies.

20 D1. A method of producing a Zika virus vaccine, comprising
(i) passaging a Zika virus on Vero cells, thereby producing a culture supernatant comprising the Zika virus;
(ii) harvesting the culture medium of (i);
(iii) precipitating the harvested culture medium of (ii), thereby producing a Zika virus
25 supernatant; and
(iv) optimally inactivating the Zika virus in the Zika virus supernatant of (iii) thereby producing an inactivated Zika virus.

30 D2. The method of claim D1, further comprising concentrating the culture medium of (ii) prior to step (iii).

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D3. The method of claim D1 or D2, wherein the precipitating of (iii) comprises contacting the culture medium of (ii) with protamine sulfate or benzoate.

D4. The method of any one of claims D1-D3, further comprising (v) dialyzing the
5 inactivated Zika virus of (iv), thereby producing a dialyzed Zika virus.

D5. The method of claim D4, further comprising (vi) filtering the dialyzed Zika virus of (v).

10 D6. The method of any one of claims D1-D5, wherein the inactivating is by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.

D7. The method of claim D6, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for at least 4 days.

15

D8. The method of claim D6 or D7, wherein the chemical inactivation agent comprises formaldehyde.

D9. The method of any one of claims D6-D8, wherein the chemical activation is
20 performed at about +4°C or about +22°C.

D10. The method of claim D8 or D9, further comprising neutralizing the formaldehyde.

D11. The method of claim D10, wherein the neutralizing is performed with sodium
25 metabisulfite.

E1. The use of the optimally inactivated Zika virus vaccine of any one of claims A1-A15 for the treatment and prevention of a Zika virus infection.

30

E2. The use of claim E1, wherein the inactivated Zika virus vaccine is administered in a first dose of a therapeutically effective amount to a subject in need thereof.

E3. The use of claim E2, wherein the inactivated Zika virus vaccine is administered in a second dose of a therapeutically effective amount to the subject.

5 E4. The use of claim E3, wherein the second dose of the inactivated Zika virus vaccine is administered about 7 days after the first dose of the Zika virus vaccine.

E5. The use of claim E3, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

10

E6. The use of claim E3, wherein the second dose of the Zika virus vaccine is administered about 28 days after the first dose of the Zika virus vaccine.

E7. The use of any one of claims E1-E6, wherein the vaccine administration results in
15 production of Zika virus neutralizing antibodies.

F1. A pharmaceutical composition for use in the treatment and prevention of a Zika virus infection, wherein said pharmaceutical composition comprises the optimally inactivated Zika
20 virus vaccine of any one of claims A1-A15.

F2. The pharmaceutical composition of claim F1, wherein the inactivated Zika virus vaccine is administered in a first dose of a therapeutically effective amount to a subject in need thereof.

25

F3. The use of claim F2, wherein the inactivated Zika virus vaccine is administered in a second dose of a therapeutically effective amount to the subject.

F4. The use of claim F3, wherein the second dose of the inactivated Zika virus vaccine is
30 administered about 7 days after the first dose of the Zika virus vaccine.

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F5. The use of claim F3, wherein the second dose of the Zika virus vaccine is administered about 14 days after the first dose of the Zika virus vaccine.

F6. The use of claim F3, wherein the second dose of the Zika virus vaccine is
5 administered about 28 days after the first dose of the Zika virus vaccine.

F7. The use of any one of claims F1-F6, wherein the vaccine administration results in production of Zika virus neutralizing antibodies.

CLAIMS

What is claimed is:

1. A Zika virus vaccine comprising an optimally inactivated Zika virus particle, wherein the Zika virus particle is able to seroconvert a subject that is administered the Zika virus vaccine with at least a 70% probability.
2. The Zika virus vaccine of claim 1, wherein the Zika virus particle is able to seroconvert the subject that is administered the Zika virus vaccine with at least a 80%, 85%, 90%, or 95% probability, preferably a 80% probability.
3. The vaccine of claim 1 or 2, wherein the Zika virus particle has a RNA genome corresponding to the DNA sequence provided by any one of the nucleic acid sequences of SEQ ID NOs: 1-11, or a variant nucleic acid sequence that is at least 88% identical to any one of SEQ ID NOs: 1-11 and able to pack a virulent Zika virus.
4. The vaccine of any one of claims 1-3, wherein the Zika virus particle has an E protein selected from the amino acid sequences provided by any one of SEQ ID NOs: 12-67, or a variant amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 12-67 and able to pack a virulent Zika virus.
5. The vaccine of any one of claims 1-4, wherein the Zika virus is inactivated by chemical inactivation, thermal inactivation, pH inactivation, or UV inactivation.
6. The vaccine of claim 5, wherein the chemical inactivation comprises contacting the Zika virus with a chemical inactivation agent for longer than is required to completely inactivate the Zika virus as measured by plaque assay.
7. The vaccine of claim 6, wherein the chemical inactivation comprises contacting the Zika virus with formaldehyde.
8. The vaccine of claim 7, wherein the formaldehyde inactivation comprises contacting the Zika virus with formaldehyde for between 2-10 days.

9. The vaccine of any one of claims 5-8, wherein the chemical activation is performed at about +4°C or about +22°C.
- 5 10. The vaccine of any one of claims 1-9, further comprising an adjuvant.
11. The vaccine of claim 10, wherein the adjuvant is an aluminum salt adjuvant.
12. The vaccine of claim 11, wherein the aluminum salt adjuvant is aluminium hydroxide
10 or aluminium phosphate salt.
13. The vaccine of any one of 10-12, wherein the vaccine comprises or further comprises an adjuvant comprising a peptide and a deoxyinosine-containing immunostimulatory oligodeoxynucleic acid molecule (I-ODN).
- 15 14. The vaccine of claim 13, wherein the peptide comprises the sequence KLKL5KLK (SEQ ID NO: 68) and the I-ODN comprises oligo-d(IC)13 (SEQ ID NO: 69).
15. The vaccine of any one of claims 1-14, further comprising one or more
20 pharmaceutically acceptable excipient.

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ABSTRACT

Described herein are Zika virus vaccines and compositions and methods of producing and administering said vaccines to subjects in need thereof.

5

Figure 1

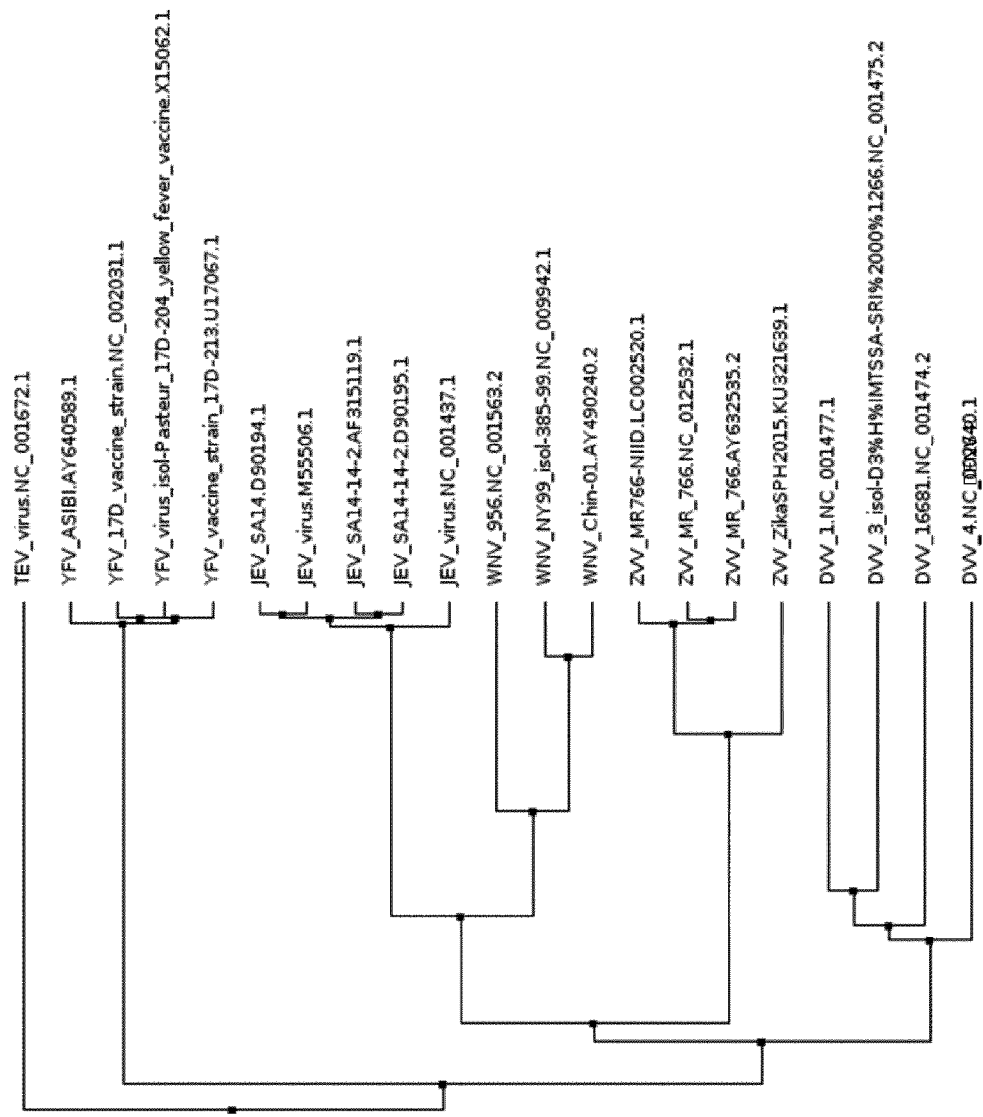


Figure 2

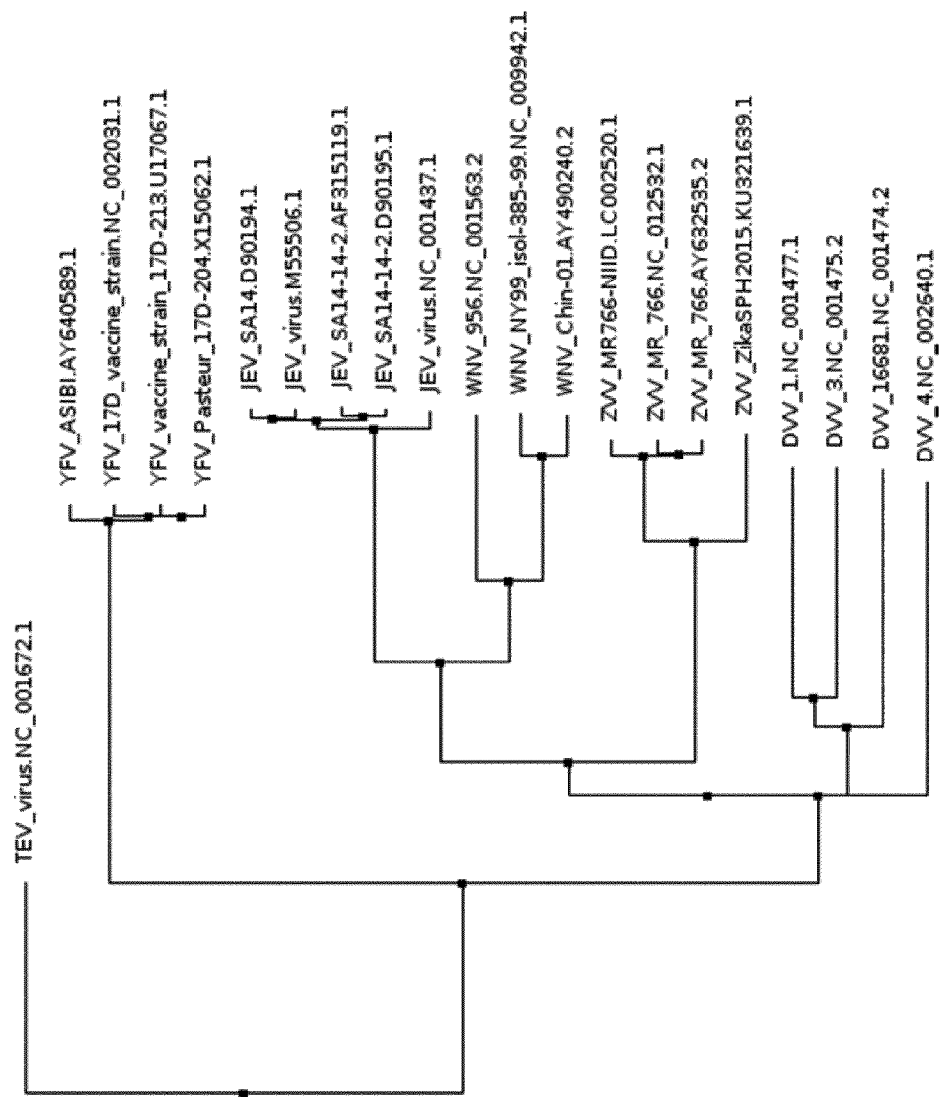


Figure 3

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
DVV_1 NC_001477.1	0	100.0	70.1	73.0	67.8	60.5	60.4	60.5	60.5	60.7	55.8	60.6	60.3	60.6	58.0	57.9	57.9	62.3	62.3	62.3	61.3
DVV_16681 NC_001474.2	1	70.1	100.0	70.8	68.6	60.5	60.5	60.5	60.6	60.7	54.9	60.9	60.3	60.4	58.3	58.3	58.3	62.7	62.8	62.8	62.2
DVV_3 NC_001475.2	2	73.0	70.8	100.0	68.9	60.2	60.1	60.1	60.1	60.1	55.8	60.3	60.3	60.1	57.5	57.6	57.5	62.6	62.7	62.7	62.5
DVV_4 NC_002640.1	3	67.8	58.6	68.9	100.0	60.5	60.6	60.6	60.4	55.4	60.8	60.3	60.5	60.5	58.0	57.9	57.9	62.1	62.0	62.0	62.1
JEV_SA14-14-2 AF315119.1	4	60.5	60.5	60.2	60.5	100.0	99.8	99.4	99.5	98.1	56.5	70.6	70.3	70.3	57.9	58.0	58.0	61.9	61.8	61.8	61.4
JEV_SA14-14-2 D90195.1	5	60.4	60.5	60.1	60.6	99.8	100.0	99.4	99.5	98.2	56.6	70.6	70.4	70.3	57.9	58.0	57.9	61.9	61.8	61.8	61.5
JEV_SA14.D90194.1	6	60.5	60.5	60.1	60.6	99.4	99.4	100.0	99.8	98.6	56.6	70.8	70.5	70.4	58.0	58.0	58.0	61.9	61.8	61.8	61.6
JEV_virus.M55506.1	7	60.5	60.6	60.1	60.6	99.5	99.5	99.8	100.0	98.6	56.7	70.8	70.4	70.3	58.0	58.1	58.0	61.9	61.8	61.8	61.6
JEV_virus NC_001437.1	8	60.7	60.7	60.1	60.4	98.1	98.2	98.6	98.6	100.0	56.6	70.6	70.5	70.6	58.3	58.3	58.4	62.1	62.0	62.0	61.7
TEV_virus NC_001672.1	9	55.8	54.9	55.8	55.4	56.5	56.6	56.6	56.7	56.6	56.0	56.2	56.7	56.6	56.9	57.0	57.0	56.6	56.7	56.7	56.7
WNV_956 NC_001563.2	10	60.6	60.9	60.3	60.8	70.6	70.6	70.8	70.8	70.6	56.2	100.0	79.6	79.6	58.1	58.0	58.1	62.4	62.3	62.3	62.2
WNV_Chin-01.AY490240.2	11	60.3	60.3	60.0	60.3	70.3	70.4	70.5	70.4	70.5	56.7	79.6	100.0	95.3	58.2	58.2	58.2	62.0	62.1	62.1	62.7
WNV_NY99_isol-385-99 NC_009942.1	12	60.6	60.4	60.1	60.5	70.3	70.3	70.4	70.3	70.6	56.6	79.6	95.3	100.0	57.8	57.8	57.8	62.0	62.0	62.0	62.4
YFV_17D_vaccine_strain_NC_002031.1	13	58.0	58.3	57.5	58.0	57.9	57.9	58.0	58.0	58.3	56.9	58.1	58.2	57.8	100.0	99.4	99.9	58.5	58.5	58.5	58.2
YFV_ASIBI.AY640589.1	14	57.9	58.3	57.6	57.9	58.0	58.0	58.0	58.1	58.3	57.0	58.0	58.2	57.8	99.4	100.0	99.4	58.4	58.4	58.4	58.1
YFV_Pasteur_17D-204.X15062.1	15	57.9	58.3	57.5	57.9	58.0	57.9	58.0	58.0	58.4	57.0	58.1	58.2	57.8	99.9	99.4	100.0	58.4	58.4	58.4	58.2
YFV_vaccine_strain_17D-213.U17067.1	16	58.0	58.3	57.5	57.9	58.0	58.0	58.0	58.1	58.3	57.0	58.2	58.2	57.8	99.9	99.4	99.9	58.4	58.4	58.4	58.1
ZVV_MR766.NIID.LC002520.1	17	62.3	62.7	62.6	62.1	61.9	61.9	61.9	61.9	62.1	56.6	62.4	62.0	62.0	58.5	58.4	58.4	100.0	99.7	99.7	88.9
ZVV_MR_766.AY632535.2	18	62.3	62.8	62.7	62.0	61.8	61.8	61.8	61.8	62.0	56.7	62.3	62.1	62.0	58.5	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_MR_766 NC_012532.1	19	62.3	62.8	62.7	62.0	61.8	61.8	61.8	61.8	62.0	56.7	62.3	62.1	62.0	58.5	58.4	58.4	99.7	100.0	100.0	88.8
ZVV_ZikaSPH2015.KU321639.1	20	61.3	62.2	62.5	62.1	61.4	61.5	61.6	61.6	61.7	56.7	62.2	62.7	62.4	58.2	58.1	58.2	88.9	88.8	88.8	100.0

60 % Identity and higher

70 % Identity and higher

80 % Identity and higher

Figure 4

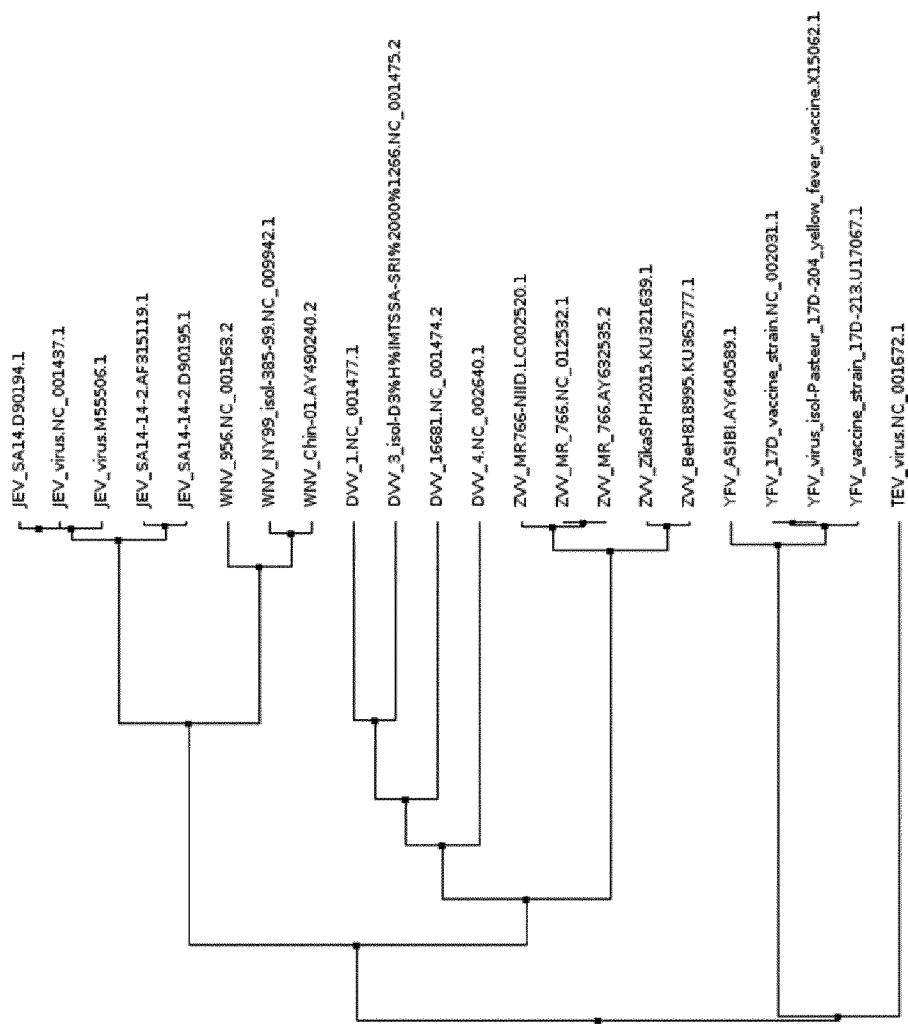


Figure 5

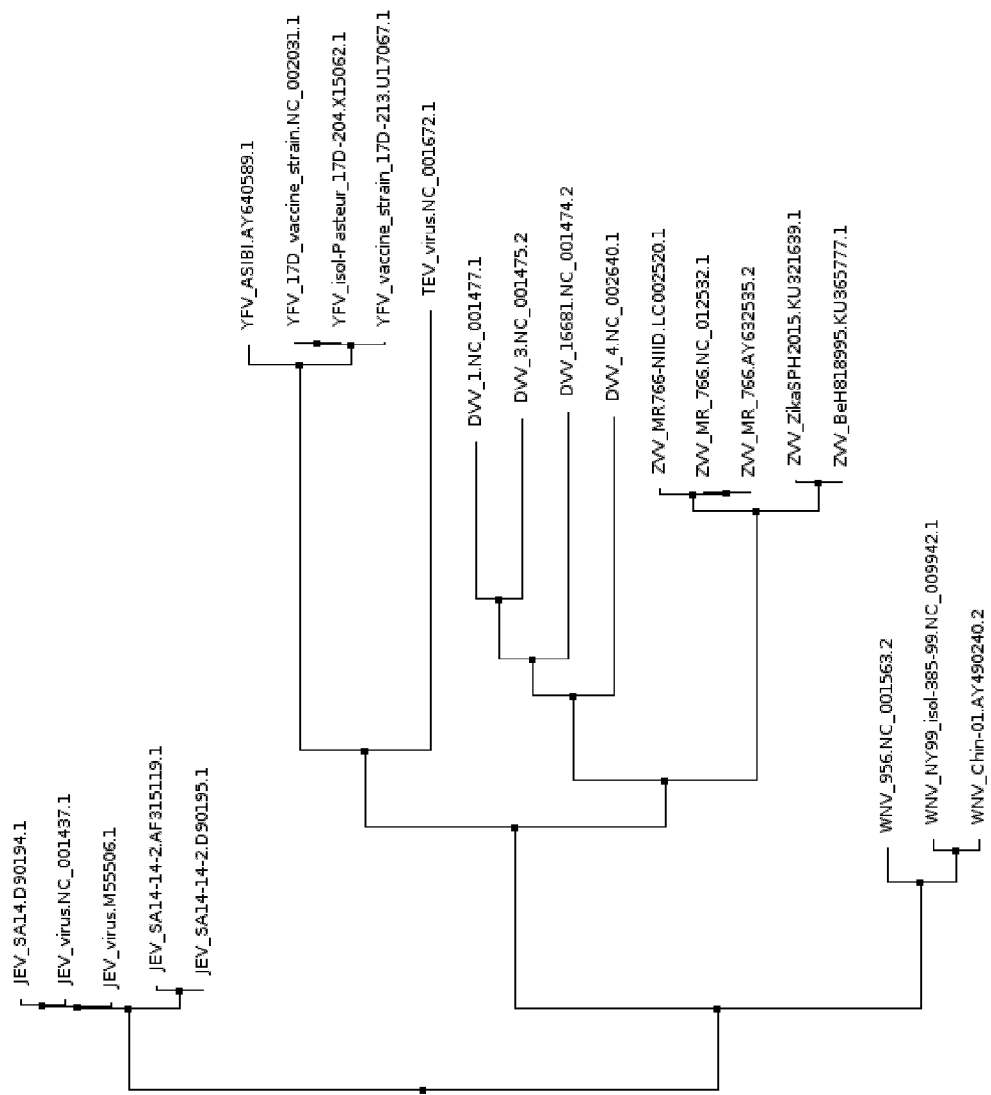


Figure 6

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
DVV_1_NC_001477.1	0	100.0	68.7	77.4	63.8	49.7	49.7	50.1	49.9	50.5	38.3	51.5	50.5	50.9	43.0	43.8	43.0	43.2	57.3	57.9	58.4	58.4	57.1
DVV_16881_NC_001474.2	1	68.7	100.0	68.7	64.0	46.7	47.1	46.9	47.5	38.9	48.4	47.6	47.6	47.6	43.6	44.4	43.6	43.6	53.6	53.8	54.2	54.2	53.8
DVV_3_NC_001475.2	2	77.4	68.7	100.0	62.8	48.0	48.0	48.2	48.6	38.2	46.8	46.7	46.3	41.6	42.2	41.6	41.7	57.3	57.7	58.2	58.2	57.1	
DVV_4_NC_002640.1	3	63.8	64.0	62.8	100.0	47.5	47.5	47.7	47.5	48.1	39.7	49.6	49.6	49.8	40.0	40.6	40.0	40.0	55.8	56.4	56.4	56.4	55.8
JEV_SA14-14-2_AF315119.1	4	49.7	46.7	48.0	47.5	100.0	99.8	98.0	98.6	97.8	39.6	76.1	76.5	76.7	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV_SA14-14-2_D90195.1	5	49.7	46.7	48.0	47.5	99.8	100.0	98.2	98.8	98.0	39.8	76.3	76.7	76.9	43.1	42.9	43.1	43.1	54.0	54.8	54.8	54.8	54.0
JEV_SA14_D90194.1	6	50.1	47.1	48.2	47.7	98.0	98.2	100.0	99.4	99.4	40.0	77.5	77.8	78.0	43.3	43.3	43.3	43.3	54.4	55.4	55.2	55.2	54.4
JEV_virus_M55506.1	7	49.9	46.9	48.2	47.5	98.6	98.8	99.4	100.0	99.2	40.0	77.3	77.6	77.8	43.3	43.3	43.3	43.3	54.2	55.2	55.0	55.0	54.2
JEV_virus_NC_001437.1	8	50.5	47.5	48.6	48.1	97.8	98.0	98.4	99.2	100.0	40.4	78.0	78.4	79.6	43.5	43.5	43.5	43.5	54.8	55.4	55.2	55.2	54.8
TEV_virus_NC_001672.1	9	38.3	38.9	38.2	39.7	39.6	39.8	40.0	40.0	40.4	100.0	41.3	41.6	41.0	42.3	41.9	42.3	42.3	39.9	39.9	39.6	39.6	39.9
WNV_956_NC_001563.2	10	51.5	48.4	46.8	49.6	76.1	76.3	77.5	77.3	78.0	41.3	100.0	94.6	94.0	43.8	43.6	43.8	43.8	55.0	55.0	54.8	54.8	55.0
WNV_Chin-01_AY490240.2	11	50.5	47.6	46.7	49.6	76.5	76.7	77.8	77.6	78.4	41.6	94.6	100.0	93.8	43.9	43.5	43.9	43.9	54.4	54.2	54.0	54.0	54.4
WNV_NY99_Isol-385-99_NC_009942.1	12	50.9	47.6	46.3	49.8	76.7	76.9	78.0	77.8	78.6	41.0	94.0	98.8	100.0	43.9	43.5	43.9	43.9	54.0	53.8	53.8	53.8	54.0
YFV_17D_vaccine_strain_NC_002031.1	13	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	100.0	97.6	100.0	99.8	42.0	43.4	43.5	43.5	42.0
YFV_ASIBIAY640589.1	14	43.8	44.4	42.2	40.6	42.9	42.9	43.3	43.3	43.5	41.9	43.6	43.5	43.5	97.6	100.0	97.6	97.4	42.4	43.8	43.9	43.9	42.4
YFV_Isol-Pasteur_17D-204_XJ5062.1	15	43.0	43.6	41.6	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	100.0	97.6	100.0	99.8	42.0	43.4	43.5	43.5	42.0
YFV_vaccine_strain_17D-213_U17067.1	16	43.2	43.6	41.7	40.0	43.1	43.1	43.3	43.3	43.5	42.3	43.8	43.9	43.9	99.8	97.4	99.8	100.0	42.0	43.4	43.5	43.5	42.0
ZVV_BeH818995_KU365777.1	17	57.3	53.6	57.3	55.8	54.0	54.0	54.4	54.2	54.8	39.9	55.0	54.4	54.0	42.0	42.4	42.0	42.0	100.0	96.8	96.4	96.4	99.8
ZVV_MR766-NIID_LC002520.1	18	57.9	53.8	57.7	56.4	54.8	54.8	55.4	55.2	55.4	39.9	55.0	54.2	53.8	43.4	43.8	43.4	43.4	96.8	100.0	98.8	98.8	96.6
ZVV_MR_766_AY632535.2	19	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.0	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	96.4	98.8	100.0	100.0	96.2
ZVV_MR_766_NC_012532.1	20	58.4	54.2	58.2	56.4	54.8	54.8	55.2	55.0	55.2	39.6	54.8	54.0	53.8	43.5	43.9	43.5	43.5	96.4	98.8	100.0	100.0	96.2
ZVV_ZikaSPH2015_KU321639.1	21	57.1	53.8	57.1	55.8	54.0	54.0	54.4	54.2	54.8	39.9	55.0	54.4	54.0	42.0	42.4	42.0	42.0	99.8	96.8	96.2	96.2	100.0

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% Identity and higher

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% Identity and higher

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% Identity and higher

